# Proceedings of the Tenth Annual Aquatic Toxicity Workshop:
November 7-10, 1983
Halifax, Nova Scotia

# Compte rendu des communications du dixième atelier annuel sur la toxicité aquatique :
du 7 au 10 novembre 1983
Halifax, Nouvelle-Écosse

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P. G. Wells and/et R. F. Addison

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The organizing committee thanks the following groups for their support: Department of Environment, EPS, Atlantic Region, Department of Fisheries and Oceans, Offshore Operators Division of the Canadian Petroleum Association, Imperial Oil (Canada) Ltd., Toronto.

Many persons worked to make the Workshop a success: Chairmen of the sessions, staff of the EPS Toxicity Section, and staff of the Marine Ecology Laboratory. We also extend our gratitude to staff of the Lord Nelson Hotel, Halifax, for their cooperation and efforts for the workshop. We also thank the authors and participants for their contributions, which made the Workshop a success.

A special thanks is due to Dr. Vincent Brown (Australia) for his thoughtful, informative and very entertaining dinner talk at the Banquet.

The proceedings were completed with the support of the Environmental Protection Service, Environment Canada, Ottawa, and the Fish Habitat Management Branch, Department of Fisheries and Oceans, Ottawa.

## PREFACE

This report is the Proceedings of the Tenth Annual Aquatic Toxicity Workshop, held in Halifax, Nova Scotia, from November 7-11, 1983.

The Aquatic Toxicity Workshop is one of a continuing series of annual workshops in Canada on aquatic and environmental toxicology, covering topics from the principles of aquatic toxicology to applications in environmental effects monitoring, setting of toxicity criteria in regulations and guidelines, and the development of water quality objectives. The Workshop emphasizes an informal exchange of ideas and knowledge on the topic among interested persons from industry, governments, consulting firms and universities. The Workshop provides an annual focus in Canada on the principles and approaches in aquatic toxicology, and the role of aquatic toxicology in the prevention and control of water pollution.

The Workshop is run by an incorporated National Steering Committee, and the proceedings are published annually with the support of the Department of Fisheries and Oceans.

Papers and posters were solicited on topics relating to research in aquatic toxicology, but with an emphasis on the following (not priorized): Environmental effects of agricultural and forestry practices; Marine ecotoxicology - principles and practice; Acid rain - current research; Use of sublethal toxicity tests for water pollution control; and Environmental contaminants - assessment and control.

About eighty papers were presented either in oral or in poster sessions. Twenty-one papers are compiled here in full, and the rest are presented as abstracts topics covered a number of major areas:

Arctic and Offshore, Statistics and Data Management, Toxicity and pH, Biochemical Toxicology, Metal Toxicology, Organohalogens and Environmental Physiology, and Various Topics.

## EDITOR'S COMMENTS

This volume contains the papers, abstracts of papers, summaries of other meetings, and summary papers that were presented at the Workshop, together with author and subject indexes and a list of participants.

The submitted papers and abstracts are published as received, and were not subjected to the initially planned external review. Comments on any aspect of the contributions should be directed to the authors.

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De nombreuses personnes ont contribué au succès de notre atelier: les présidents de séance, l'équipe de la Section de la toxicité du SPE, ainsi que l'équipe du Laboratoire d'écologie marine. De même, nous exprimons notre gratitude à l'équipe du Lord Nelson Hotel d'Halifax pour sa coopération et les efforts qu'elle a déployés pour la réalisation de cet atelier. Enfin, nous remercions de leur contribution les auteurs et participants qui ont été le facteur essentiel du succès de l'atelier.

Les comptes rendus ont été rédigés avec l'aide du Service de la protection de l'environnement, Environnement Canada, Ottawa et de la Direction de la gestion de l'habitat du poisson, ministère des Pêches et des Océans, Ottawa.

Nous tenons à remercier tout spécialement M. Vincent Brown (Australie) pour l'allocution à la fois intéressante, instructive et très agréable qu'il a prononcée au dîner.

## AVANT PROPOS

Le présent rapport est le compte rendu du dixième atelier annuel sur la toxicité aquatique, qui s'est tenu à Halifax (Nouvelle-Écosse), du 7 au 11 novembre 1983.

L'atelier sur la toxicité aquatique fait partie d'une série d'ateliers annuels, tenus au Canada, sur la toxicité aquatique et environnementale, traitant de sujets allant des principes des critères de toxicité en toxicologie aquatique aux applications à la surveillance des effets environnementaux, l'établissement des règlements et lignes directrices, et le développement des objectifs en matière de qualité des eaux. L'atelier consiste essentiellement en un échange spontané d'idées et de connaissances entre des personnes intéressées venant de l'industrie, des gouvernements, de firmes de consultation et d'universités. L'atelier permet de rassembler chaque année les points de vue des différents organismes canadiens sur les principes et méthodes de toxicologie aquatique, ainsi que sur le rôle de la toxicologie aquatique dans la prévention et la lutte contre la pollution des eaux. L'atelier est conduit par un comité de direction national, et ses comptes rendus sont publiés annuellement avec l'aide du ministère des Pêches et des Océans.

Les articles et affiches demandés étaient centrés sur des sujets relatifs à la recherche en toxicologie aquatique, particulièrement dans les domaines suivants (sans priorité): les effets environnementaux des pratiques agricoles et forestières, l'écotoxicologie marine (principes et pratiques), les pluies acides (recherches actuelles), les tests de toxicité sublétale en vue du contrôle sublétale en vue du contrôle de la pollution des eaux, et les contaminants environnementaux (évaluation et contrôle).

Environ quatre-vingt communications ont été présentées, soit oralement, soit en session d'affiches. Vingt et une communications ont été rassemblées dans leur intégralité, le reste est présenté sous forme de résumé. Parmi les domaines qui ont été abordés, citons: Arctique et haute mer, statistique et gestion des données, toxicité et pH, toxicologie biochimique, toxicologie des métaux, organohalogènes et physiologie environnementale, et divers autres sujets.

## COMMENTAIRES DE L'ÉDITEUR

Dans ce volume, on trouvera les communications, les extraits de communications, les résumés d'autres réunions, ainsi que les communications condensées qui ont été présentées à l'atelier, un index des auteurs et des sujets et une liste des participants.

Les communications et extraits qui nous ont été remis sont publiés dans l'état où nous les avons reçus, et n'ont pas été soumis à la révision externe précédemment prévue. Tout commentaire sur ces contributions doit être adressé aux auteurs.

On peut obtenir les comptes rendus de cet atelier, ainsi que de ceux qui l'ont précédé, auprès des organismes suivants:

| | | |
|---|---|---|
| Micromedia Limited<br>144, rue Front ouest<br>Toronto, (Ontario)<br>M5J 2L7 | et | Président permanent<br>Ateliers sur la toxicité aquatique<br>Direction de la gestion de l'habitat du poisson<br>Ministère des Pêches et des Océans<br>200, rue Kent<br>Ottawa (Ontario)<br>K1A 0E6 |

# OPENING SESSION

R.F. Addison, Chairman

# THE THREEFOLD PATH

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BRINKHURST, R.O. 1985. The threefold path. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 3-9.

The advantages and disadvantages of toxicology and field surveys as approaches to environmental assessment and management are compared. It is suggested that simple toxicological procedures provide a useful forensic tool for effluent monitoring and management but that field surveys are required to assess the effectiveness of controls based on simple parameters. The combination of legislation, toxicology and ecology combine effectively for at least three separate situations in environmental management.

BRINKHURST, R.O. 1985. The threefold path. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 3-9.

On compare les avantages et les désavantages de la toxicologie et des études sur le terrain comme méthodes d'évaluation et de gestion environnementale. Il résulte de cette comparaison que des méthodes toxicologiques simples fournissent un outil légal utile permettant de surveiller et de gérer les effluents, mais que les études sur le terrain sont nécessaires pour évaluer l'efficacité des contrôles basés sur des paramètres simples. L'association de la législation, de la toxicologie et de l'écologie se révèle efficace dans au moins trois situations distinctes de gestion environnementale.

## INTRODUCTION

I have long been an opponent of undue reliance on toxicity testing to achieve environmental assessment and control in aquatic ecosystems for a series of reasons that will be detailed below. Until recently I declined to attempt such work but, in order to improve credibility in this debate, my colleagues and I recently made use of the opportunity to carry out a comparative series of simple toxicity tests using a number of species of aquatic oligochaetes. The species were selected to represent as wide a spectrum of field-established tolerances to general classes of pollutants as possible. The worms were exposed to a series of individual contaminants and environmental factors both singly and in combinations of factors, and both pure and mixed worm cultures were employed. Sublethal stress was then detected using respiration measurements. As these studies have been or are about to be published (Brinkhurst et al 1983; Chapman and Brinkhurst, 1984; Chapman et al 1982a, b, c.), the results will not be laboured here, except to point out that they show that the rank-orders of species tolerances to classes of pollutants established from field work were generally substantiated, but that individual LC50 values could be shifted by between a half and forty-eight times the initial values by quite small variations in environmental conditions. There is no such thing as a laboratory-determined LC50 that can predict the survival of a species in the field in the face of the full suite of biotic and abiotic variables that confront it as a continuing kaleidoscope of stress and competition. While my knowledge of the overwhelming body of toxicological literature is fragmentary, I know of no other set of comparative multi-species, multifactorial experimentation with which to compare it but I have referred many times to a very persuasive example of single species versus trifactorial stress and this serves to illustrate that such results are to be regarded as normal (Wuhrmann and Woker, 1955). Most of what I have to say can also be found in a series of papers on biological monitoring by a variety of authors published by Water Research in 1981-1982.

If this result is so easy to demonstrate, why then are so many of the workers in the pollution field still wedded to the toxicity concept? Forced to concede that toxicology will not go away, I am persuaded to assess the situation again, and to seek a compromise posture that would at least afford those of my persuasion a more visible place in the ranks of those determined to prevent avoidable assaults on natural ecosystems. This will be presented in the form of a series of debating points, to which many other points could undoubtedly be added.

### Problem

We are aware of environmental damage in aquatic ecosystems due to human activity such as:

A. Physical alteration (e.g. dams, canals, drainage, diversion, resource extraction).
B. Waste disposal (heat, organic wastes, toxic chemicals, inert suspended material).
C. Use and misuse of products (pesticides, detergents, fertilizers).

### Requirement

We therefore need ways to determine the source of damage, the degree of damage, and the causative agent where there are unknown. Once identified, we need a scientifically based body of control legislation.

Thirdly, we need methods for the evaluation of the degree of conformity to regulations and the efficacy of the levels of controls applied in each instance in order to achieve environmental integrity.

## Scientific Response

We have adopted three sets of responses to this situation:

A. The massive descriptive overkill of impact assessment before the fact with its lack of focused prediction of actual hazards (discussed by Rosenberg et al 1981).
B. The detection of ecological integrity by field scientists, using comparisons of pre- and post-impact or upstream/downstream comparisons.
C. Estimation of toxicity of wastes by laboratory scientists.

## Difficulties

Legislation tends to be designed around effluent standards matched to toxicity estimation rather than the end result of the degree of ecological change produced in the receiving environment. Hence the laboratory approach tends to be the dominating influence in all spheres of pollution biology in Canada.

## The Ecosystem Analysis Pro and Con:

A. Pro. The method

1. is site specific.
2. uses simple, cheap sampling methods (though skilled technicians should be employed).
3. takes account of quality of the receiving water due to "upstream" discharges and integrates the effect of multiple point-sources or mixed discharges.
4. can be used in cheap initial assessments of nature, source and extent of damage, especially in smaller urban drainage systems where these are unknown.
5. can be used to detect source, nature and damage from single incidents as well as chronic discharges.
6. can be predictive if enough cause/effect descriptions become available.
7. can be objective if ordination and clustering and similar statistical tools are employed.
8. provides a direct test of the original need for habitat protection of real ecosystems.
9. can provide objective tests of the efficacy of indirect control measures through effluent standards.

B. Con. The method

1. can lead to excessive descriptive reports if analyses of all microhabitats are investigated rather than one or two representative microhabitats.
2. requires a good taxonomic base.
3. becomes harder to use as physical sampling problems increase in largescale "wilderness" ecosystems.
4. requires a basic understanding of regional seasonal cycles in order to avoid multiple surveys each year.
5. has seldom been used as a predictive tool.
6. is considered costly and time consuming.

In the same way, static bioassays with standard target species such as rainbow trout, Daphnia or fathead minnows can be evaluated as follows:

**Toxicology Pro and Con:**

A. Pro. Laboratory studies ......

1. yield repeatable results under controlled conditions.
2. fit the reductionist approach and produce "quantative" data recognizable by chemists, physiologists and engineers.
3. are similar in approach to industrial product screening with laboratory animals (pharmaceuticals, cosmetics, etc.).
4. employ carefully bred and maintained laboratory strains of animals of known genetic lineage proved standard targets.
5. can demonstrate toxicity of materials relative to a standard toxicant, and produce a predictive toxicology.

B. Con. Laboratory studies ......

1. use standard laboratory animals not present in most receiving waters, so that test results may be considerred irrelevant to specific situations in court.
2. do not account for natural variability in biotic and abiotic factors in the real world which render laboratory data inapplicable to the determination of contaminant effects on receiving waters.
3. omit subtle sublethal effects and effects on non-adult stages of the life cycle that are not detected in short-term bioassays.
4. are simplistic in that most effluents contain mixtures of contaminants, and the proportions usually vary with time and from plant to plant within each industrial group.

Some may suggest that recent developments have obviated some or all of these defects, and the programme before us this week shows how such consideration is being given to sub-lethal stress, physiological mechanisms and improvements in methods. In situ methods may be thought to overcome many of my "con" arguments, for instance, but they are still subject to criticism if they ignore the endemic, on-going toxicity tests being performed in the ecosystem itself. Taking a carefully controlled laboratory in a trailer into the field is a very expensive proposition, and while on-site studies do allow a fair test of an effluent which may change during transportation to a home based laboratory, other advantages are not too clear. The use of endemic species may be compromised by the lack of control over the genetic background of the test species or lack of comparative data. While there is no time to get deeply involved in a debate about the methodology of toxicity testing, the basic point I wish to make is that without reference back to the receiving ecosystem to ensure the efficacy of our control systems, we have no way of evaluating our progress in mitigating environmental damage. While field methods may still require some degree of diagnostic skill akin to that used in evaluation of human health, advances in statistical procedures can remove much of the old elitist subjective nature of this approach.

## DISCUSSION

As in all human ventures, cooperative efforts employing all available tools where they are most appropriate produce the best result. The following prospectus appeals to me, and undoubtedly the reader will be able to elaborate on the theme.

### Pre-Development Impact Assessment

Field work should be used to establish major pool sizes and flux rates among the major species associations present. The major "players" are identified as being those responsible for the most material and the most action in the system. Some species might be important for the structure they provide, for example, even if relatively static in energy flow terms. These studies should be used to make best possible predictive statements of probable significant impacts of the proposed human endeavour, not lists of every conceivable pertubation, or worse, catalogues of every conceivable organism that can be detected without reference to the associated hazards.

The impact of specific contaminants associated with the project should be predicted from previous comparable situations, reinforced by toxicology using standard reference animals relative to previously-investigated contaminants of similar chemical structure. Comparative toxicology on the key organisms from the specific site can be used to improve predictive capability.

### Post-Impact Assessment of Environmental Damage

Field surveys of selected microhabitats (riffles in trout streams, for example) determine source and extent of damage and general class of contaminant where unknown. Absence of species expected in such habitats, coupled with chemical analyses, will be most persuasive diagnostic factors when supported by toxicological tests on the relevant species and contaminants used in appropriate experimental conditions.

### Monitoring Known Point Sources

Known effluents can best be regulated by simple standards as contravention can readily be demonstrated. These standards are best based on toxicological parameters or traditional tests like the B.O.D. The level of stringency imposed needs to be established in relation to the existing ecological condition of the receiving water as well as downstream uses. Periodic assessment of the ecological condition of the receiving water will ensure that the permitted discharge levels are not damaging the ecosystem in some unforeseen manner even if the regulations are being faithfully complied with.

## CONCLUSION

The ultimate test of our protective measures is the survival of a viable complex of living organisms in the field. There is only one way that can be properly assessed, and that is by investigating the natural system itself. In our enthusiasm for piling up data on the effect of A on B in our neatly controlled laboratories, let us not forget the need to improve our training of field biologists in the universities and our need to support the provision of taxonomic guides to the flora and fauna of Canada. These are needed in order to upgrade the effectiveness of the field-oriented corner of the triangle otherwise based on regulations and laboratory studies.

While I must applaud the initiative that brings together so many of you in these annual gatherings, I look forward to some increase in contacts between the applied aquatic field biologists that are members of the North American Benthological Society (for example) and this audience, for the benefit of both.

## ACKNOWLEDGEMENTS

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# ARCTIC AND OFFSHORE

Mike Hutcheson, Chairman

# SENSITIVITY OF ARCTIC MARINE AMPHIPODS AND FISH TO PETROLEUM HYDROCARBONS

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CARLS, M.G. and S. KORN. 1985. Sensitivity of arctic marine amphipods and fish to petroleum hydrocarbons. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 11-26.

We determined the sensitivities of six circumpolar benthic species to water-soluble fractions (WSF) of Cook Inlet crude oil and naphthalene in separate tests. The species tested were the amphipods Anonyx nugax, Boeckosimus nanseni, and Gammaracanthus loricatus, a mysid, Mysis relicta, Arctic cod (Boreogadus saida), and a sculpin (Oncocottus hexacornis). Exposures were flow-through and lasted up to 40 days. Median lethal concentrations (CL50's) of the WSF ranged from 1.6 to 3.8 ppm total aromatics. Naphthalene assays were conducted at several temperatures (1.5 to 9.6°C) to study temperature effects on sensitivity to hydrocarbons. Naphthalene LC50's ranged from 1.35 to 3.35 ppm. General relationships between exposure temperatures and LC50's were not found. In the absence of toxicants, upper lethal temperatures for the crustaceans were surprisingly high: 17-24°C, suggesting the assay temperatures in themselves were not particularly stressful.

We compared the sensitivities of these Arctic marine species to the sensitivities of temperate species previously tested at this laboratory (ABL) using the same flow-through procedures and toxicants, and evaluated two alternative hypotheses: 1) marine Arctic animals, are adapted to a wide range of environmental parameters, and therefore are unusually resistant to unaccustomed stresses such as petroleum hydrocarbons, or 2) marine Arctic animals are unusually sensitive to hydrocarbon stress because they are already stressed to their limits by the environment in which they live. We conclude that Arctic species are about equal in sensitivity to temperate species. However, their habitat is more vulnerable to the effects of petroleum hydrocarbon pollution than temperate habitats because low temperatures lead to slower losses of hydrocarbons from volatilization and biodegradation, and oil entrapment under sea ice can result in very lengthy exposures.

CARLS, M.G. et S. KORN. 1985. Sensitivity of arctic marine amphipods and fish to petroleum hydrocarbons. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 11-26.

Nous avons déterminé la sensibilité de six espèces benthiques circumpolaires aux fractions solubles dans l'eau du pétrole brut et du naphtalène de l'inlet Cook, au moyen de tests séparés. Les espèces testées ont été les amphipodes Anony nugax, Boeckosimus nanseni et Gammaracanthus loricatus, un myside, Mysis relicta, le saida franc (Bereogadus saida) et un chabot (Oncocottus hexacornis). Les expositions ont été faites à flot direct, et ont duré jusqu'à 40 jours. Les concentrations létales médianes (CL50) des produits

solubles dans l'eau ont varié entre 1,6 et 3,8 ppm, pour les produits aromatiques totaux. Les essais sur le naphtalène ont été conduits à diverses températures (de 1,5 à 9,6 °C) afin d'étudier les effets de la température sur la sensibilité aux hydrocarbures. Les CL50 du naphtalène ont varié entre 1,35 et 3,35 ppm. Il n'a pas été trouvé de relation générale entre les températures d'exposition et le CL50. En l'absence de polluants toxiques, les plus fortes températures létales, pour les crustacés, ont été surprenantes: 17 à 24 °C; ce qui indique que par elles-mêmes, les températures d'essai n'étaient pas particulièrement stressantes.

Nous avons comparé la sensibilité de ces espèces marines arctiques à celle d'espèces tempérées précédemment testées dans notre laboratoire (ABL) avec les mêmes méthodes à flot direct et les mêmes produits toxiques, et nous avons évalué deux hypothèses différentes: 1) les animaux marins arctiques sont adaptés à une gamme étendue de paramètres environnementaux, et sont par conséquent extrêmement résistants à des stress inhabituels comme ceux des hydrocarbures du pétrole, ou 2) les animaux marins arctiques sont exceptionnellement sensibles au stress des hydrocarbures parce qu'ils sont déjà stressés jusqu'à leurs limites extrêmes par l'environnement dans lequel ils vivent. Nous concluons que les espèces arctiques présentent une sensibilité relativement égale à celle des espèces tempérées. Cependant, leur habitat est plus vulnérable aux effets de la pollution des hydrocarbures pétroliers que ne le sont les habitats tempérés, car les températures basses conduisent à une diminution des pertes d'hydrocarbures par volatilisation et par biodégradation, et l'emprisonnement du pétrole par la glace de l'eau de mer peut entraîner des expositions persistantes.

## INTRODUCTION

Increasing oil exploration and production in the Beaufort Sea area of the Alaskan and Canadian Arctic has increased the probability of oil spills in this environment. This area is physically vulnerable to oil pollution because 1) the shelf areas of the Beaufort Sea are very shallow, so spills are likely to contaminate both pelagic and benthic habitats, 2) cold temperatures reduce the loss of petroleum hydrocarbons by volitilization and biodegradation, and 3) ice cover can trap oil for long periods of time. Arctic habitats are biologically vulnerable because of low productivity, low species diversity, and slow rates of biological recovery (Dunbar 1968; Wacasey 1975).

Nearshore Arctic marine animals live in a very harsh environment compared to temperate species: they may experience unusually broad temperature fluctuations, salinity changes, and photoperiod extremes. For example, Craig and Haldorson (1979) reported seasonal variations in the shallow waters (up to 3m) of Simpson Lagoon, near Prudhoe Bay, Alaska, of:

| | Spring* | Summer* | Fall* | Winter* |
|---|---|---|---|---|
| temperature | 0-5°C | 7-10°C | 0-6°C | -2-0°C |
| salinity | 1-10°/oo | 18-25°/oo | 18-25°/oo | 26-60°/oo |

Daily salinity and temperature values can change rapidly: Truett (1978) commonly observed changes up to 6°C and 15°/oo in Simpson Lagoon.

These widely fluctuating environmental conditions (temperature, salinity, and photoperiod) suggest two alternative hypotheses concerning the physiological vulnerability of Arctic marine animals: 1) Arctic animals are adapted to a wide range of environmental parameters and therefore are unusually resistant to unaccustomed stresses such as petroleum hydrocarbons, or 2) Arctic animals are unusually sensitive to hydrocarbon stress because they are already stressed to their limits by the environment in which they live.

The objective of our study was to test the sensitivities of several Arctic species to the water-soluble-fraction (WSF) of Cook Inlet oil, and compare them to the sensitivies of temperate species previously tested in our laboratory with the same flow-through procedures and oil. We also used naphthalene, an important di-aromatic component in the WSF of Cook Inlet crude oil, as a reference toxicant. We have restricted our comparisons between Arctic and temperate species to experimental results collected in our laboratory in order to avoid the problems created by variations in techniques and toxicants which plague oil toxicity research.

Because the Arctic animals we studied are naturally subjected to a wide range of temperatures, we performed bioassays at several different temperatures. As ancillary data we also tested temperature tolerances without toxicants to ensure that the bioassay test temperatures were below tolerance extremes.

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* Spring = late June-early July, summer = mid July-mid August, fall = late August-September, Winter = October-early June.

## Materials and Methods

Six Arctic species were available for testing: the amphipods Boeckosimus nanseni, Gammaracanthus loricatus, and Anonyx nugax, a mysid, Mysis relicta, Arctic cod (Boreogadus saida), and a sculpin (Oncocottus hexacornis). All specimens were collected in Simpson Lagoon near Prudhoe Bay, Alaska except Anonyx nugax was collected in Auke Bay, Alaska. Collections were made in April and May 1979. All six organisms are circumpolar. Prior to experimentation, the animals were held in 800 l tanks at approximately 2°C at about 30°/oo. They were fed Oregon moist pellets[1], Tetramin, salmon roe, and chopped fish.

Flow-through dosing techniques were used (except Mysis test): the WSF of the crude oil was generated by constantly percolating water at 1 l/min through a replenished oil layer 10-20 cm deep in a 16 cm diameter X 90 cm glass cylinder (Moles et al this workshop). Oil was added to the lower surface of the slick at about 1.5 ml/min and allowed to overflow from the upper surface at the same rate. This apparatus produced 2 to 2.3 ppm oil in water, measured as total aromatic hydrocarbons. Stock solutions of naphthalene were generated by forcing water through naphthalene flakes in a 4-l flask.

Toxicant concentrations were measured daily. The concentration of aromatic oil components (Table 1) were monitored daily by gas-liquid chromatography using methods described by Moles and Rice (1983). Total oil concentrations were estimated by adding the concentrations of the mono- and dinuclear aromatic hydrocarbons. Naphthalene samples were measured spectrophotometrically at 219 nm.

Naphthalene assays were conducted at several temperatures, ranging from 1.5 - 9.6°C. Toxicants plus diluent seawater were distributed to 5-l glass aquaria or 19-l glass jars at 300 ml/min through glass tubing. Turnover times (99% replacement) were 80 min for B. nanseni and A. nugax tests and 4.5 h for all others except mysids. Mysid tests were static with daily toxicant replenishment. The number of organisms per dose ranged from 12 - 80 (Tables 2 and 3), but the biomass was kept below 3 g/l. Tests ranged from 8-day exposures plus a 3-day recovery period to 40 days (Tables 2 and 3). Mortality observations were made at 2, 4, 8, and 24 h and daily thereafter.

Acclimated and non-acclimated temperature tolerances of the crustaceans were determined in 5-l aquaria with a turnover rate of 3 h (99% replacement). A. nugax and B. nanseni controls were maintained at 8°C±0.4; G. loricatus and M. relicta controls were maintained at 4°C±0.8. The animals were fed every other day during the experiment. Acclimatization limit of the animals were determined by raising the temperatures slowly at the rate of 0.5°C±1.4 per day for 3 replicates of 20 animals. Mortality data were collected daily over a period of 41 days. Temperature tolerances of non-acclimatized animals were determined by subjecting 2 replicates of 10 animals each to sudden temperature changes; any given animal was tested only once in this manner. Rapid temperature jumps were approximately 5, 10, 15, and 20°C.

The concentrations at which half the animals died (LC50's) were determined from the data by logit analysis (Finney 1952) or Spearman-Karber analysis (Hamilton et al 1977). Correction for control mortality (Abbott 1925) was applied as necessary. The LC50 values were considered baseline (stable) when daily mortalities fell to zero, or matched control mortality rates. Differences between the LC50's were analyzed by

TABLE 1 --CHARACTERIZATION OF THE WATER-SOLUBLE FRACTION OF COOK INLET CRUDE OIL IN TESTS WITH VARIOUS ARCTIC SPECIES

| Organism | % Monoaromatic hydrocarbons | % Benzene in monoaromatic hydrocarbons | % Diaromatic hydrocarbons | % Naphthalene in diaromatic hydrocarbons |
|---|---|---|---|---|
| *Boeckosimus nanseni* | 95.6 ± 4.1<br>97.0 ± 1.6 | 49.0 ± 10.4<br>38.3 ± 13.6 | 4.4 ± 4.1<br>3.0 ± 1.6 | 55.6 ± 12.2<br>50.6 ± 11.1 |
| *Anonyx nugax* | 97.6 ± 0.6 | 50.2 ± 9.3 | 2.4 ± 0.6 | 50.3 ± 5.0 |
| *Gammarancanthus loricatus*, *Oncocottus hexacornis*, and *Boreogadus saida* | 97.1 ± 1.2 | 38.0 ± 2.6 | 2.9 ± 1.2 | 45.7 ± 7.6 |
| *Mysis relicta* | 97.4 | 53.8 | 2.6 | 55.7 |
| $\bar{X}$ | 96.9 ± 0.8 | 45.8 ± 7.3 | 3.1 ± 0.8 | 51.6 ± 4.2 |

computing support functions for two different logistic models and computing a chi square comparison (Jeff Fujioka, manuscript on file at ABL).

## RESULTS

### Comparative sensitivities

Cook Inlet crude oil baseline WSF LC50's ranged from 1.6 to 3.8 ppm total aromatic hydrocarbons (Figure 1, Table 2). Species sensitivities, arranged from the least to the most sensitive were: the amphipods *B. nanseni*, *A. nugax*, *M. relicta*, and Arctic cod. Arctic cod responded the most rapidly, reaching a stable LC50 after approximately 5 days. The sculpins and the amphipod *G. loricatus* did not respond measurably after 8 d exposures to the WSF, but these assays were not extended further.

Assays with Cook Inlet crude oil on *B. nanseni* indicate this amphipod may have been lethally damaged before mortalities appeared. Due to toxicant dosing problems one *B. naneni* assay only lasted 13 days--about half as long as the other. The LC50's in the shorter exposure became measurable after the dosing ended, and followed the same pattern as the longer exposures. However, because the mean temperatures between the two tests were different, it is difficult to be certain of the relationship between the two exposures.

Average naphthalene 8-day LC50's ranged from 1.35 ppm to 3.35 ppm (Table 3). Species sensitivities from the least to the most sensitive were: the amphipods *B. nanseni*, *G. loricatus*, and *A. nugax*, sculpins and Arctic cod. All species except *B. nanseni* (and possibly *A. nugax*) reached their baseline lethal levels by 8 days. After 40 days, the LC50 of naphthalene for the amphipod *B. nanseni* dropped to 58% of its 8 day value. Sculpin response was the most rapid, followed by Arctic cod, then amphipods *G. loricatus* and *A. nugax*; the response of *B. nanseni* was the slowest (Figure 2). Mysid responses were also rapid.

### Temperature tolerances

Acclimated median lethal upper temperatures of the crustaceans were surprisingly high: *G. loricatus* 24°C±1.0, *B. nanseni* 20.6°C±0.3, *M. relicata* 17°C±3.7, and *A. nugax* 17.2°C±0.7. Nonacclimatized median lethal temperatures were about 66% of the acclimatized temperatures: 16.5°C, 11.0°C, 14.7°C±0.2, and 9.7°C±0.2, respectively.

### Effects of temperature on sensitivity to naphthalene

General relationships between exposure temperatures (1.5 to 9.6°C) and LC50's for naphthalene were not found: LC50's tended to vary unpredictably with temperature for most species, but tended to increase with temperature for *O. hexacornis* (Table 3, Figure 3). Temperature dependent variations in sensitivity for each species were not significant at $\alpha=0.025$ except in one case: *B. nanseni* showed significantly less sensitivity at one midrange temperature (4.8°C). (Table 3 and Figure 3).

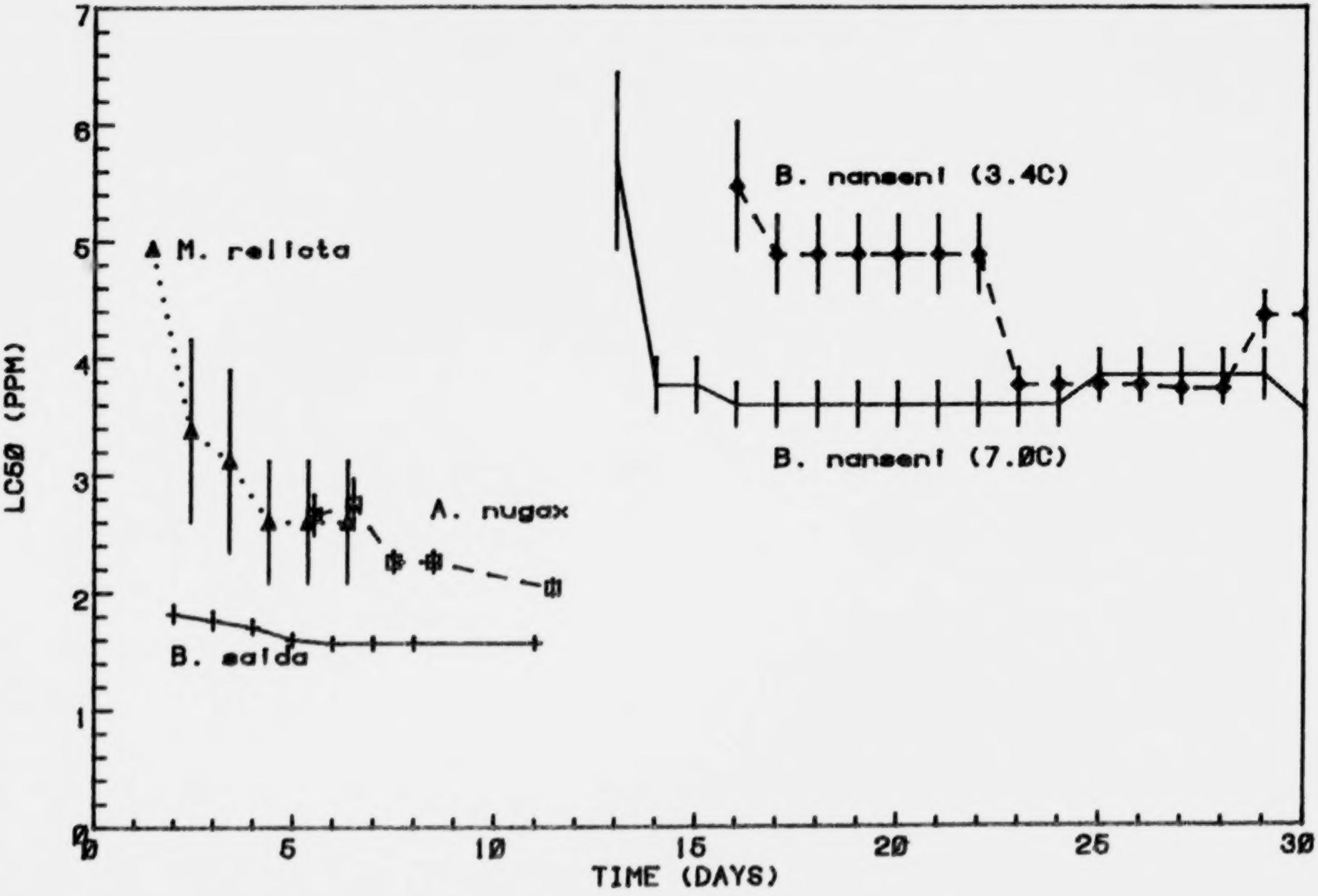

FIGURE 1 MEDIAN LETHAL CONCENTRATIONS (LC50's) OF THE WATER-SOLUBLE-FRACTION (WSF) OF COOK INLET CRUDE OIL MEASURED OVER TIME. BASELINE (stable) RESPONSES ARE WHEN VALUES BECOME ASYMPTOTIC TO THE X AXIS. ERROR BARS ARE THE 95% CONFIDENCE LIMITS

TABLE 2 SPECIES SENSITIVITIES TO THE WATER-SOLUBLE FRACTION OF COOK INLET CRUDE OIL

| | Temperature | | Baseline LC50 (ppm) | Exposure duration | |
|---|---|---|---|---|---|
| Species | °C, s | | ppm ± CI† | days | *n*/dose |
| *Boeckosimus* | 3.4, | 0.3 | 3.7 ± 1.16 | 13* | 80 |
| *nanseni* | 7.0, | 2.0 | 3.8 ± 1.66 | 27 | 60 |
| *Anonyx nugax* | 3.5, | 0.3 | 2.3 ± 0.94** | 8 | 15 |
| *Gammaracanthus loricatus* | 2.0, | 0.5 | >1.7 | 8 | 15 |
| *Oncocottus hexacornis* | 2.0, | 0.5 | >1.7 | 8 | 12 |
| *Boreogadus saida* | 2.0, | 0.5 | 1.6 ± 0.12 | 8 | 12 |
| *Mysis relicta* | 4.4 | - | 2.7 ± 0.7 | 4 | 12 |

† 95% Confidence interval
* Plus a 14-day recovery period.
** May not have reached the baseline level.

## DISCUSSION

Our findings agree with the relative species sensitivities reported elsewhere: out of 6 species tested by Foy (1979), *A. nugax* was the most sensitive arctic amphipod and *Boeckosimus* sp. was the most resistant. Percy and Mullin (1975) found sculpin fry (*Myoxocephalus quadricornis*) quite sensitive to crude-oil dispersions. Rice et al. (1979) also generally found vertebrates to be more sensitive than invertebrates to petroleum hydrocarbons.

Our observation that temperature affects survival unpredictably is consistent with findings of other researchers (Sprague 1970; Korn et al. 1979). Sensitivities were not affected uniformly by an 8°C assay temperature range. This implies temperature did not serve as an additional stress factor during our assays. The tolerances of the amphipods and mysid to warm temperatures were surprisingly high, indicating that our bioassays were well within temperature tolerance ranges, and that the assay temperatures did not particularly stress the animals. We suspect significant changes in sensitivity to petroleum hydrocarbons may only occur near temperature tolerance extremes.

The Arctic amphipods and mysid were remarkably tolerant of high temperatures. The acclimatized median lethal temperature determined for *M. relicta* (17°C) agrees with the tolerated upper extreme (17-18°C) determined by Holmquist (1959). If temperature tolerance is considered an index of environmental 'hardiness', it is apparent that all four

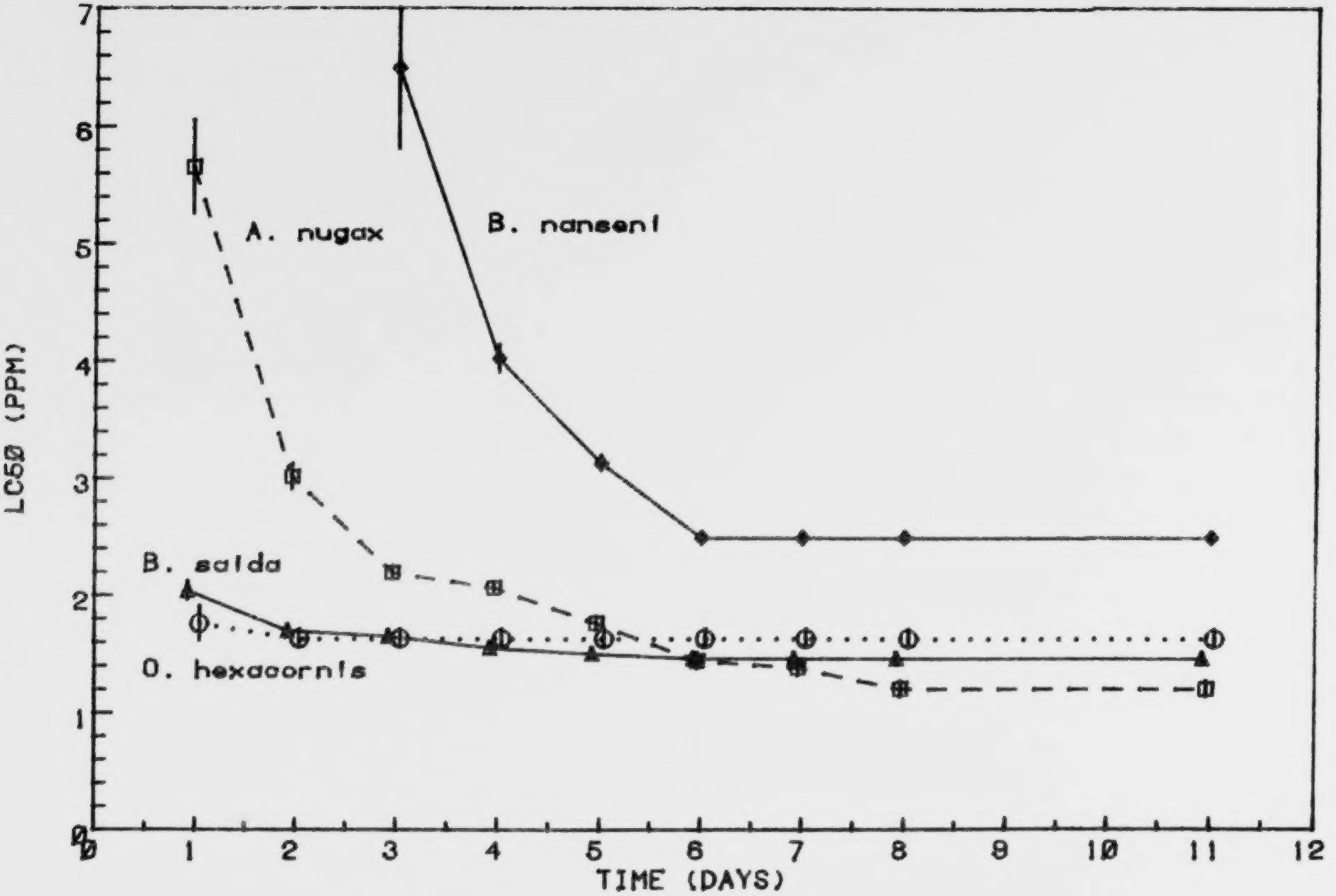

FIGURE 2 MEDIAN LETHAL CONCENTRATIONS (LC50's) MEASURED OVER TIME FOR NAPHTHALENE BIOASSAYS AT 6.4-6.9°C (mean temperature range). ONLY THOSE SPECIES TESTED AT THESE TEMPERATURES ARE PLOTTED. BASELINE (stable) RESPONSES ARE WHEN VALUES BECOME ASYMPTOTIC TO THE X-AXIS. ERROR BARS ARE THE 95% CONFIDENCE LIMITS

TABLE 3 SPECIES SENSITIVITES TO NAPHTHALENE

| | Temperature | | LC50 (ppm) | | Duration | *n* |
|---|---|---|---|---|---|---|
| Species | °C, | s | 96 h ± CI† | 8 d ± CI | (days) | dose |
| *Boeckosimus* | 4.8, | 0.5 | -- | 5.3 ± 0.44 | 8 | 15 |
| *nanseni* | 3.6, | 0.4 | -- | 3.4* | 40 | 80 |
| | 6.9, | 0.4 | 4.0 ± 0.42 | 2.5 ± 0.18 | 8 | 15 |
| | 9.6, | 0.3 | 2.9 ± 0.33 | 2.3 ± 0.34 | 8 | 15 |
| average = | 6.6, | 2.2 | 3.5 ± 7.24 | 3.4 ± 2.16 | | |
| *Anonyx nugax* | 4.8, | 0.5 | 2.7 ± 0.35 | 2.0 | 8 | 15 |
| | 6.9, | 0.4 | 2.1 ± 0.19 | 1.2 ± 0.24 | 8 | 15 |
| | 9.6, | 0.3 | 1.8 | 1.5 ± 0.24 | 8 | 15 |
| average = | 7.1, | 2.4 | 2.2 ± 1.15 | 1.6 ± 0.94 | | |
| *Gammaracanthus loricatus* | 2.0, | 0.5 | 2.3 | 2.1 | 8 | 15 |
| *Oncocottus* | 1.5, | 0.2 | 1.1 | 1.0 ± 0.22 | 8 | 12 |
| *hexacornis* | 2.0, | 0.5 | -- | 1.1 | 8 | 12 |
| | 6.4, | 1.1 | -- | 1.6 ± 0.08 | 8 | 12 |
| | 8.5, | 1.3 | 1.8 | 1.7 ± 0.14 | 8 | 12 |
| average = | 4.6, | 3.4 | 1.4 ± 0.62 | 1.4 ± 0.57 | | |
| *Boreogadus* | 1.5, | 0.2 | 1.5 ± 0.14 | 1.5 ± 0.12 | 8 | 12 |
| *saida* | 2.0, | 0.5 | 1.2 ± 0.30 | 1.2 ± 0.30 | 8 | 12 |
| | 6.4, | 1.1 | 1.6 | 1.5 ± 0.16 | 8 | 12 |
| | 8.5, | 1.3 | 1.2 ± 0.52 | 1.2 ± 0.52 | 8 | 12 |
| average = | 4.6, | 3.4 | 1.4 ± 0.28 | 1.4 ± 0.23 | | |
| *Mysis relicta* | 4.4 | - | 1.9 ± 0.4 | - - | 4 | 12 |

† 95% confidence interval.

* Average temperature after 40 d = 5.0 ± 1.8; 40 d LC50 = 1.94 ± 0.19 ppm.

species of crustaceans are quite hardy. Tencati (1970) describes *B. nanseni*, which had a midrange response, as a hardy species.

We did not find differences between 'Arctic' and 'temperate' fish and crustaceans when comparing the sensitivities determined in this study with other species tested with

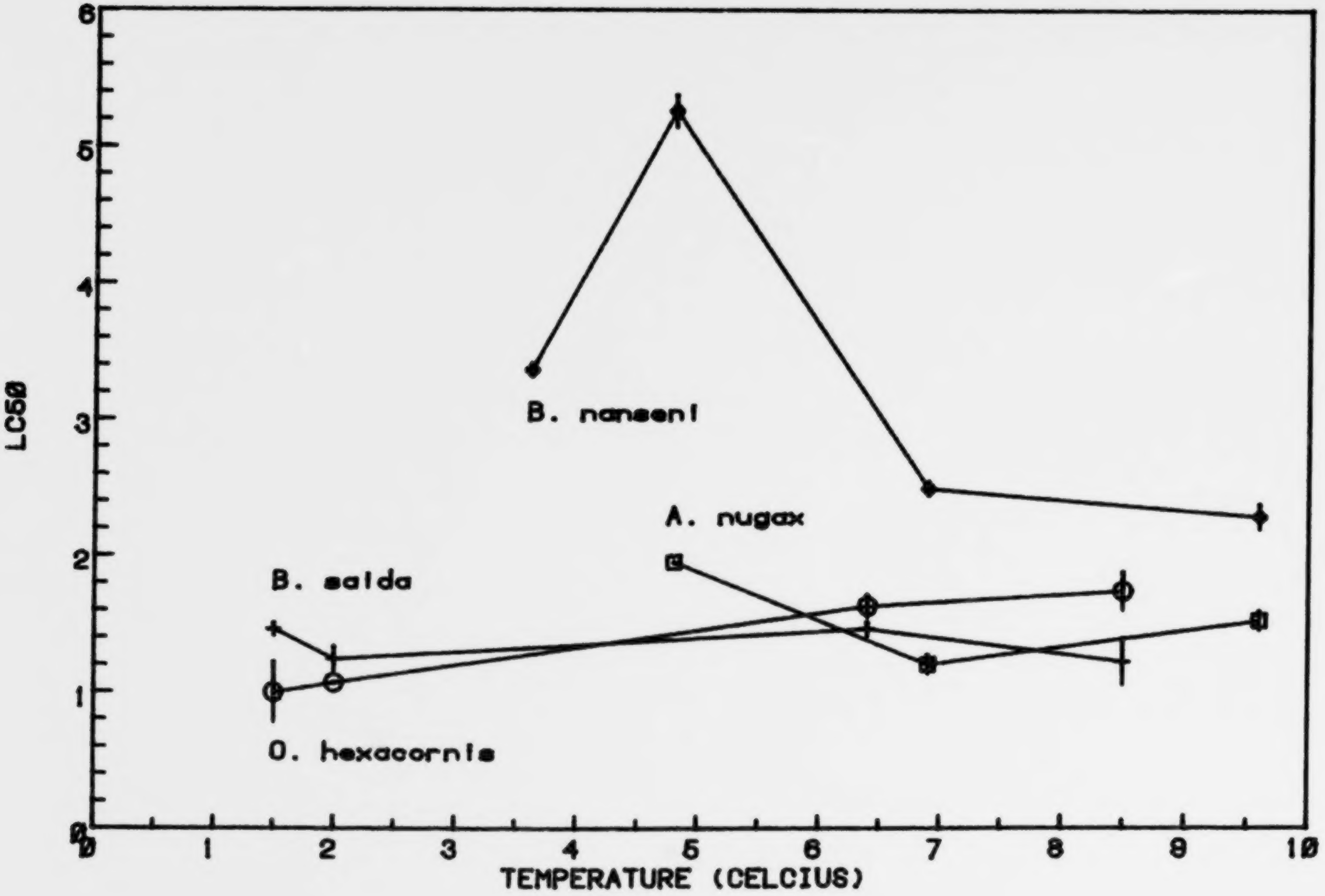

FIGURE 3 RELATIONSHIPS BETWEEN BIOASSAY EXPOSURE TEMPERATURES AND RESULTANT 8 d LC50 VALUES. A GENERAL TREND FOR ALL SPECIES DID NOT OCCUR

similar flow-through techniques at this laboratory (ABL) (Table 4). For example, naphthalene LC50's ranged from 0.8 to 2.2 for temperate crustaceans, and 1.0 to 5.7 for Arctic amphipods. The LC50's of temperate and Arctic fish exposed to the WSF of Cook Inlet crude oil range from 1.2 to about 2 ppm. This similarity is not surprising when the geographic ranges of the species tested are compared: the ranges of nearly all the 'temperate' species tested (see Table 4) extend northward along the Pacific coast of North America to at least the Bering Sea, and about half extend into the Arctic (see listing in Table 4). On the other hand, half of the 'arctic' species we tested extend southward at least as far as the Bering Sea, and two (Anonyx nugax and G. loricatus) extend into the North Pacific. Note: the mysid data were not used for these calculations because of differences in testing methodology.

TABLE 4 COMPARISONS OF 'ARCTIC' SENSITIVITY DATA (COLLECTED IN THIS STUDY) WITH 'TEMPERATE' SENSITIVITY DATA COLLECTED AT AUKE BAY LABORATORY. ALL TESTS WERE FLOW-THROUGH. COMPARISONS ARE MADE AT 8 DAYS. THE LC50 RANGES ARE FROM THE LOWER 95% CONFIDENCE BOUNDS OF THE LOW LC50 VALUES TO THE UPPER BOUNDS OF THE HIGHEST LC50 VALUES

| | WSF of Cook Inlet Crude LC50's | | naphthalene LC50's | |
|---|---|---|---|---|
| Group | temperate | Arctic | temperate | Arctic |
| fish* | 1.2 to 1.8 | 1.5 to >1.7 | 0.9 to 3.8 | 0.8 to 1.9 |
| crustaceans** | 0.5 to 1.5 | 1.3 to 5.5 | 0.8 to 2.2 | 1.0 to 5.7 |

* fish represented in the temperate data pool are: Oncorhynchus gorbuscha fry (Moles and Rice 1983; Brodersen and Rice in prep; Andrews and Rice in prep; Gharrett and Rice in prep), O. kisutch fry and smolts (Moles and Rice in prep; Moles 1980; Moles, Bates and Korn 1981; Stickle, Sabourin, and Rice 1982), and Salvelinus malma char (Thomas and Rice in prep).

** Crustaceans represented in temperate data pool are: Eualus suckleyi (Gharrett and Rice in prep; Brodersen and Rice in prep; Andrews and Rice in prep), Pandalus hypsinotus (Brodersen and Carls in prep), P. borealus (data on file at ABL), juvenile Paralithodes camtschatica (data on file at ABL), and Hemigrapsus nudus (Brodersen and Rice in prep; Gharrett and Rice in prep).

The Arctic species we tested were not unusually resistant to petroleum hydrocarbons. Arctic species sensitivities overlapped the range of 'temperate' species sensitivities to oil tested at other times in this laboratory. Adaptation to widely variable environment factors, such as temperature, salinity, and photoperiod, does not insure resistance to abnormal stress factors, such as petroleum hydrocarbons.

The cold temperatures of the Arctic have more impact on the vulnerability of the habitat than the physiological sensitivity of the animals. Arctic habitats are more vulnerable to the effects of petroleum hydrocarbon pollution than temperate habitats because low temperatures lead to slower losses of hydrocarbons from volatilization and biodegradation, and oil entrapment under sea ice can insure lengthy exposures. Once physical or chemical perturbations have caused damage to the habitat and decreases in animal populations, recovery and re-establishment of communities may be slow because of low productivity, low species diversities, and slow growth rates (Dunbar 1968; Grainger 1975; Wacasey 1975).

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Acknowledgement -- Funding support was provided by the U.S. Department of Interior's Bureau of Land Management through the Outer Continental Shelf Energy Assessment Program of the National Oceanic and Atmospheric Administration.

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## FIGURE LEGENDS

| | |
|---|---|
| Figure 1 | Median lethal concentrations (LC50's) of the water-soluble-fraction (WSF) of Cook Inlet crude oil measured over time. Baseline (stable) responses are when values become asymptotic to the x axis. Error bars are the 95% confidence limits. |
| Figure 2 | Median lethal concentrations (LC50's) measured over time for naphthalene bioassays at 6.4-6.9°C (mean temperature range). Only those species tested at these temperatures are plotted. Baseline (stable) responses are when values become asymptotic to the x-axis. Error bars are the 95% confidence limits. |
| Figure 3 | Relationships between bioassay exposure temperatures and resultant 8 d LC50 values. A general trend for all species did not occur. |

# THE IMPACT OF DRILLING-WASTE DISPOSAL ON TRACE METALS IN SCALLOP TISSUE AND SEDIMENTS NEAR SABLE ISLAND

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CARTER, J.A., S.D. MACKNIGHT, and C.W. ROSS. 1985. The impact of drilling-waste disposal on trace metals in scallop tissue and sediments near Sable Island. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 27-52.

Trace metal levels in sediments and scallop tissue from the Olympia A-12 well site near Sable Island were determined in a preliminary assessment of the impact of water-based drilling-waste discharge on the marine environment. Samples were collected immediately before and after drilling, in April 1982 and January 1983, respectively.

Post-drilling sediments showed no apparent accumulation of clay-sized particles characteristic of drilling muds. Metal levels in both pre- and post-drilling sediments were low compared to levels in texturally-equivalent Bay of Fundy sediments. Zinc and cadmium had relatively high potential bioavailabilities (ratio of weak acid-leachable metal concentration to total metal concentration).

Barium, copper, and mercury, possibly associated with the barite discharge, showed post-drilling accumulations in sediments 0.5 nautical miles downcurrent from the well site. An accumulation of weak acid-leachable chromium, associated with the chrome lignite discharge, was detected east of the well site. However, the significant metal accumulations were generally limited to 2-3 fold increases over pre-drilling levels in sediments at individual stations. Mercury showed a 20 x increase in post-drilling sediments south of the well site.

With the exception of zinc, which was uniformly distributed in scallop tissue, all metals were more concentrated in the viscera than in the adductor muscle. There was apparent accumulation of chromium and zinc in scallop tissue at the well site and several nautical miles north and west of the well site. This suggested the influence of sacrificial zinc anodes at the well site and mud discharge at the more remote sites.

There was no correlation of trace metal levels in scallop tissue with those in the sediments. These preliminary data suggested the initial wide dispersal of the lighter fractions of drilling-waste during discharge, more limited dispersal of barite, and subsequent reworking in the bottom sediments in the direction of the residual current.

CARTER, J.A., S.D. MACKNIGHT, and C.W. ROSS. 1985. The impact of drilling-waste disposal on trace metals in scallop tissue and sediments near Sable Island. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 27-52.

Le niveau des métaux à l'état de trace dans les sédiments et le tissu des pétoncles du puits de forage Olympia A-12 près de l'île de Sable a été déterminé dans une évaluation préliminaire des effets du déversement des rejets de forage à base d'eau sur l'environnement marin. Des échantillons ont été rassemblés immédiatement avant et après forage, en avril 1982 et en janvier 1983, respectivement.

Les sédiments d'après forage n'ont montré aucune accumulation apparente de particules de la taille des argiles caractéristiques des boues de forage. Les niveaux des métaux dans les sédiments avant et après forage ont été bas, par comparaison aux niveaux dans les sédiments de structure équivalente de la baie de Fundy. Le zinc et le cadmium ont présenté des biodisponibilités potentielles relativement élevées (rapport entre la concentration de métaux lixiviables par des acides faibles et la concentration métallique totale).

Le baryum, le cuivre et le mercure, peut-être associés avec le déversement de baryte, ont présenté des accumulations après forage dans les sédiments à 0,5 mille marin en aval du lieu de forage. Une accumulation de chrome lixiviable par des acides faibles, associée au déversement de lignite à chrome, a été détectée à l'est du lieu de forage. Cependant, les accumulations importantes de métal ont été généralement limitées à des accroissements de 2 ou 3 fois par rapport aux niveaux avant forage dans les sédiments aux stations individuelles. La concentration de mercure était 20 fois plus importante dans les sédiments après forage au sud du lieu de forage.

A l'exception du zinc qui était réparti uniformément dans le tissu des pétoncles, tous les métaux étaient plus concentrés dans les viscères que dans le muscle adducteur. Il n'y avait pas d'accumulation apparente de chrome et de zinc dans le tissu des pétoncles à l'endroit du forage, ni à plusieurs milles marins au nord et à l'ouest. Ce fait laisserait supposer l'influence des anodes de zinc sacrifiées au lieu de forage et du déversement des boues aux endroits plus éloignés.

On n'a pas trouvé de corrélation entre le niveau des métaux à l'état de trace dans le tissu des pétoncles et celui qui a été observé dans les sédiments. Ces données préliminaires indiqueraient une dispersion initiale étendue des fractions plus légères des rejets de forage pendant le déversement, une dispersion plus limitée de la baryte, et une redistribution consécutive des sédiments de fond dans la direction du courant résiduel.

# INTRODUCTION

Offshore drilling involves disposal of drilling muds, fluids, and well cuttings, collectively referred to as drilling-waste, into the marine environment. Trace metals occurring in the drilling-waste may accumulate in bottom sediments and fauna within several kilometres of a well site (Crippen et al., 1980; EG and G Environmental Consultants, 1982). The physical state of the receiving environment is an important factor in the final fate of drilling-waste. There is apparently little accumulation of metals in bottom sediments in dynamic locations (Houghton et al., 1980).

Exploration and delineation drilling off Canada's east coast have increased significantly in recent years. However, trace metal concentrations near offshore well sites in this area have not been monitored in the past. This report is a preliminary assessment of the impact of drilling-waste disposal on trace metal levels in sediments and fauna at the Olympia A-12 well site near Sable Island, Nova Scotia.

## The Well Site

The Olympia A-12 well site is located several kilometres north of the East Spit of Sable Island (Fig. 1) in 48 m of water. The surficial sediments in this area are well-sorted sands. The long-term residual current at the well site flows to the east (Mobil Oil Canada Ltd., 1983).

The well site was occupied by the jack-up rig Zapata Scotian from April 17, 1982 to January 11, 1983. At least the following amounts of drilling-waste, in addition to mud and cuttings, were discharged during the drilling program (Dresser-Magcobar, unpublished data):

barite: 2,720 tonnes
Cromex®: 135 tonnes (chrome lignite)
Resinex®: 48 tonnes (synthetic rosin)
chrome-free lignosulphonate: 27 tonnes

This waste was a potential source of barium and chromium, in addition to contaminants such as lead, zinc, cadmium, copper, and mercury.

## Materials and Methods

### Sampling

The sampling stations at Olympia A-12 are shown in Figure 1. Water depth and sediment type at each station are noted in Table 1. Station 1 was adjacent to the well site. The other stations were at 0.5, 1.0, and 2.0 nautical miles on the main compass axes. The terminal points on the south and east axes could not be sampled because of turbulent water near Sable Island.

Pre-drilling sampling was conducted from the M.V. Brandal between April 14 and 18, 1982. Surficial sediment samples were collected at each station with a Van Veen grab.

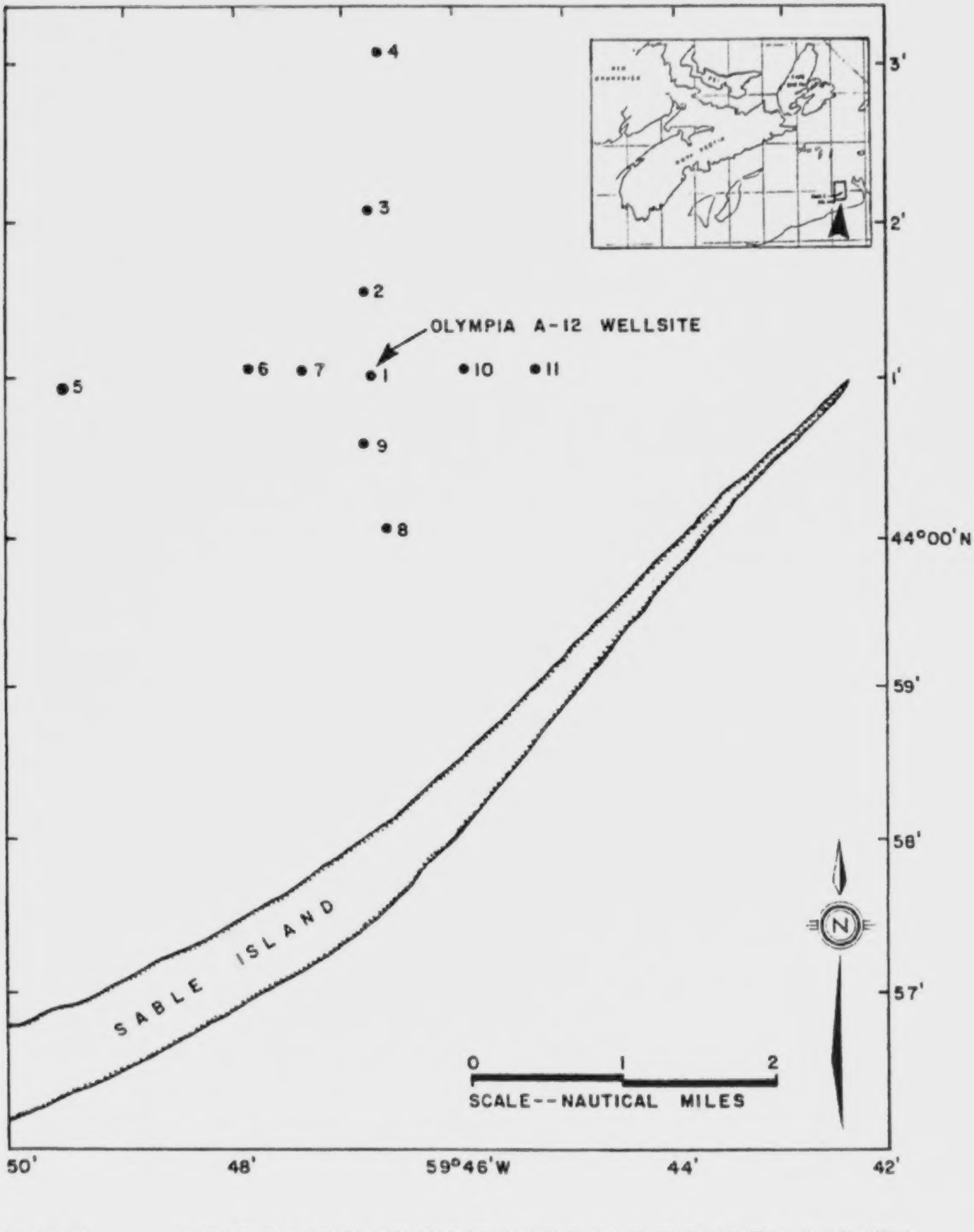
OLYMPIA A-12 WELLSITE
1
2
3
4
5
6
7
8
9
10
11
SABLE ISLAND
0
1
2
SCALE--NAUTICAL MILES
N
3'
2'
1'
44°00'N
59'
58'
57'
50'
48'
59°46'W
44'
42'

FIGURE 1 LOCATION OF SAMPLING STATIONS AT THE OLYMPIA A-12 WELL SITE

TABLE 1 CHARACTERISTICS OF SAMPLING STATIONS AT THE OLYMPIA A-12 WELL SITE (see Fig. 1 for locations)

| Station | Depth (m) | Sediment Type (pre-drilling) | Sediment Type (post-drilling) |
|---|---|---|---|
| 1 | 48 | 100% sand | -[1] |
| 2 | 49 | 100% sand | 100% sand |
| 3 | 44 | 100% sand | - |
| 4 | 55 | 0-0.3% gravel;<br>96.7-100% sand;<br>0-2% silt; 0-1% clay | 0.3-8% gravel;<br>92-99.7% sand |
| 5 | 44 | 0.6-2% gravel;<br>99.4-98% sand | - |
| 6 | 46 | 100% sand | - |
| 7 | 46 | 0.2-4% gravel;<br>96-99.8% sand | 100% sand |
| 8 | 38 | 2-3% gravel;<br>97-98% sand | - |
| 9 | 42 | 0.2-0.6% gravel;<br>99.4-99.8% sand | 0.3-0.9% gravel;<br>99.1-99.7% sand |
| 10 | 48 | 96-100% sand;<br>0-3% silt; 0-1% clay | - |
| 11 | 48 | 94-97% sand;<br>2-3% silt; 1-3% clay | 94-100% sand;<br>2-3% silt;<br>2-3% clay |

[1] not analyzed

Plastic spoons were used to obtain subsamples from the centre of each grab. Samples were stored frozen in plastic bags. A fine-mesh Hessler-Sanders sledge proved inadequate for collection of fauna. The experience of sampling during the first cruise led to modifications in the second cruise.

Post-drilling sampling was conducted from the M.V. Must 'N' Tell on January 23, 1983, 12 days after the rig left the site. Three surficial sediment samples (individual grabs) were collected at each station with a neoprene-sealed Van Veen grab and subsampled in the same manner used previously. Five grab samples were also collected from a sandy control station in 44 m of water, northwest of Sable Island (44°04'N; 60°30'W) and about 30 nautical miles west of Olympia A-12. Bottom fauna were collected at each station with a scallop rake. Fauna were depurated in ambient seawater for at least 24 hours, rinsed, and frozen whole.

### Chemical Analysis

Sediments and sea scallops (Placopecten magellanicus) were analyzed for trace metals. All sediments were microwave oven-dried, lightly ground, and bottled. Sediments were subjected to both a weak acid-leach (25% strength acetic acid) (Engler et al., 1977) and to total dissolution using a hydrofluoric acid/aqua regia mixture (Loring and Rantala, 1977). Organic and inorganic carbon content were determined using the LECO furnace method. Particle size distribution was determined by standard sieve and pipette techniques (Plumb, 1981).

The individual scallops were dissected into adductor muscle and remaining viscera after thawing. Wet and dry (15 hours at <50°C) weights of tissues were determined. The dried samples were then wet-digested with Ultrex-grade nitric acid and hydrogen peroxide.

Trace metal determinations were made with a Perkin-Elmer 372 atomic absorption spectrophotometer in either flame, flameless (ramped HGA 2100), or cold vapour mode. Quality control was monitored by concurrent analysis of reference sediments (BCSS-1, MESS-1, USNBS Estuarine Sediment) and tissue (USNBS Oyster Tissue). Standards for calibration purposes were prepared using similar matrices.

### Analysis of Data

Means, standard deviations, and 95% confidence intervals were determined for all sediment and scallop data on a per-station basis. Significant differences between pre- and post-drilling metal concentrations in sediments at each station and between metal concentrations in scallop tissues at all stations after drilling were determined by confidence interval analysis (Natrella, 1972). The sample sizes upon which the data were based are summarized in Table 2. Because scallop size was determined to have a significant influence on trace metal concentrations in tissues (Carter and MacKnight, 1983), only the data from moderate-size scallops (adductor muscle dry weight between 1.6 and 5.0 g; mean shell height of 12.8 + 2.7 cm) are reported here.

## RESULTS

### Sediments

Analysis of sediments in post-drilling samples showed no significant changes in particle size distribution in the survey area compared to pre-drilling data (Table 1). All samples comprised at least 92% well-sorted sand (500 - 700 µm median particle size) and no more than 6% silt and clay. The average organic carbon content of sediments was 1 mg/g.

TABLE 2 SAMPLE SIZES USED IN THE DETERMINATION OF 95% CONFIDENCE INTERVALS

| | | Sediments | | Scallops | |
|---|---|---|---|---|---|
| Station | | Total Metals | Weak Acid-Leachable Metals | Adductor Muscle | Viscera |
| 1 | (pre-drilling) | 2 | 2 | | |
| | (post-drilling) | 3 | 3 | 26 | 26 |
| 2 | (pre-drilling) | 2 | 2 | | |
| | (post-drilling) | -[1] | 3 | 3 | 3 |
| 3 | (pre-drilling) | 4 | 4 | | |
| | (post-drilling) | 3 | 3 | 1 | 1 |
| 4 | (pre-drilling) | 1 | 1 | | |
| | (post-drilling) | 3 | 3 | 14 | 14 |
| 5 | (pre-drilling) | 2 | 2 | | |
| | (post-drilling) | 1 | 1 | 17 | 17 |
| 6 | (pre-drilling) | 2 | 2 | | |
| | (post-drilling) | 1 | 1 | 3 | 3 |
| 7 | (pre-drilling) | 2 | 2 | | |
| | (post-drilling) | - | 3 | | |
| 8 | (pre-drilling) | 2 | 2 | | |
| | (post-drilling) | 1 | 1 | 2 | 2 |
| 9 | (pre-drilling) | 2 | 2 | | |
| | (post-drilling) | 3 | 3 | | |
| 10 | (pre-drilling) | 2 | 2 | | |
| | (post-drilling) | 3 | 3 | 2 | 2 |
| 11 | (pre-drilling) | 2 | 2 | | |
| | (post-drilling) | - | 3 | | |
| Control (post-drilling) | | 2 | 2 | | |

[1] not analyzed

Table 3 shows the ranges of mean concentrations of trace metals in sediments at Olympia A-12 before and after drilling, and at the control site. Of the metals analyzed (total dissolution), barium was the most concentrated, followed, in descending order, by chromium, zinc, lead, copper, mercury, and cadmium. Weak acid-leachable metal concentrations in sediments were low.

TABLE 3 RANGES OF MEAN CONCENTRATIONS OF TRACE METALS IN SURFICIAL SEDIMENTS IN THE OLYMPIA A-12 SURVEY AREA AND AT THE CONTROL (in ppm)

| | Stations | | |
|---|---|---|---|
| Metal | Pre-drilling | Post-drilling | Control |
| Barium | | | |
| Total | 56.6 - 211.3 | 32.5 - 296.3 | 147.0 |
| Weak acid-leachable | 0.1 - 1.5 | 0.2 - 1.5 | 3.3 |
| Chromium | | | |
| Total | 1.7 - 32.9 | 2.7 - 22.9 | 12.2 |
| Weak acid-leachable | 0.5 - 1.3 | 0.5 - 1.7 | 0.5 |
| Zinc | | | |
| Total | 1.4 - 14.6 | 1.7 - 11.9 | 6.0 |
| Weak acid-leachable | 0.5 - 6.9 | 0.4 - 9.7 | 5.2 |
| Lead | | | |
| Total | 1.5 - 6.5 | 2.8 - 7.9 | 8.9 |
| Weak acid-leachable | 0.05 - 0.68 | 0.06 - 0.91 | 0.39 |
| Copper | | | |
| Total | 0.39 - 1.54 | 1.05 - 2.79 | 1.79 |
| Weak acid-leachable | 0.03 - 0.22 | 0.03 - 0.15 | 0.12 |
| Mercury | | | |
| Total | 0.01 - 0.14 | < 0.01 - 0.23 | 0.01 |
| Weak acid-leachable | < 0.01 | < 0.01 | < 0.01 |
| Cadmium | | | |
| Total | 0.03 - 0.10 | 0.02 - 0.08 | 0.05 |
| Weak acid-leachable | < 0.01 - 0.02 | < 0.01 - 0.02 | < 0.01 |

Weak acid-leachable metal is assumed to be biologically available (Luoma and Jenne, 1976). The ratio of weak acid-leachable metal concentration to total metal concentration is therefore a measure of the potential bioavailability of metals in sediments. Of the metals analyzed in this study, zinc showed the highest potential bioavailability (34.2 - 86.7%), followed by cadmium, copper, lead, and chromium (Table 4). Barium showed very low potential bioavailability.

Figs. 2 - 14 show the mean trace metal concentrations in sediments at the Olympia A-12 well site before and after drilling. Weak acid-leachable mercury is not shown because it was below the limit of detection. There were only a few significant increases in metal concentrations in sediments after drilling. These are summarized below:

TABLE 4 RATIOS OF WEAK ACID-LEACHABLE METAL CONCENTRATION TO TOTAL METAL CONCENTRATION (%), BASED ON OVERALL MEAN CONCENTRATIONS FOR SETS OF PRE-DRILLING, POST-DRILLING, AND CONTROL STATIONS

| | Stations | | |
|---|---|---|---|
| Metal | Pre-drilling | Post-drilling | Control |
| Zinc | 34.2 | 45.5 | 86.7 |
| Cadmium | < 25.9 | < 35.3 | 20.0 |
| Copper | 11.6 | 6.1 | 6.7 |
| Lead | 4.7 | 4.7 | 4.4 |
| Chromium | 5.7 | 8.6 | 4.1 |
| Barium | 0.4 | 0.5 | 2.2 |

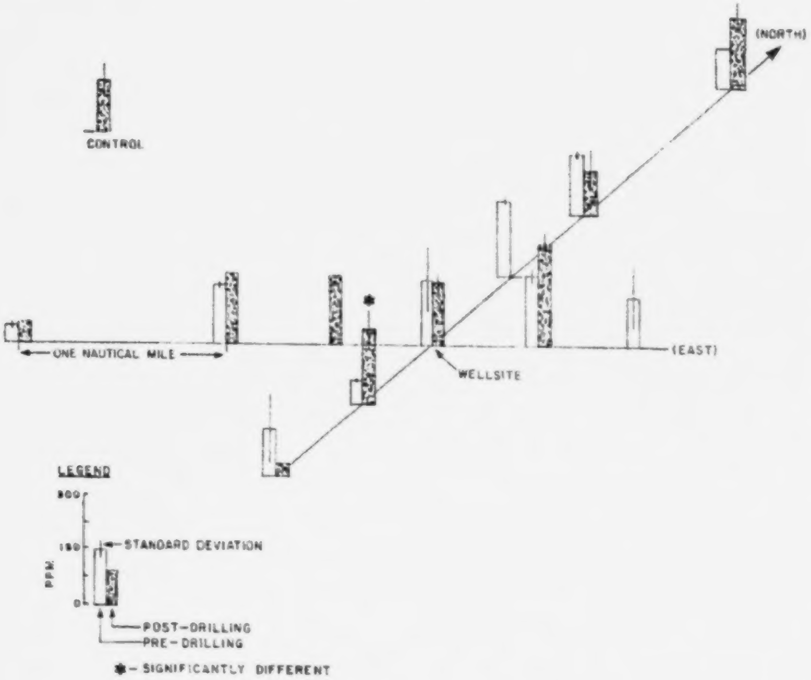

FIGURE 2 TOTAL BARIUM IN SURFICIAL SEDIMENTS AT OLYMPIA A-12

Station 9: Apparent accumulation of total and weak acid-leachable barium, total copper, and total mercury in post-drilling sediments.

Station 10: Apparent accumulation of weak acid-leachable barium and total copper in post-drilling sediments.

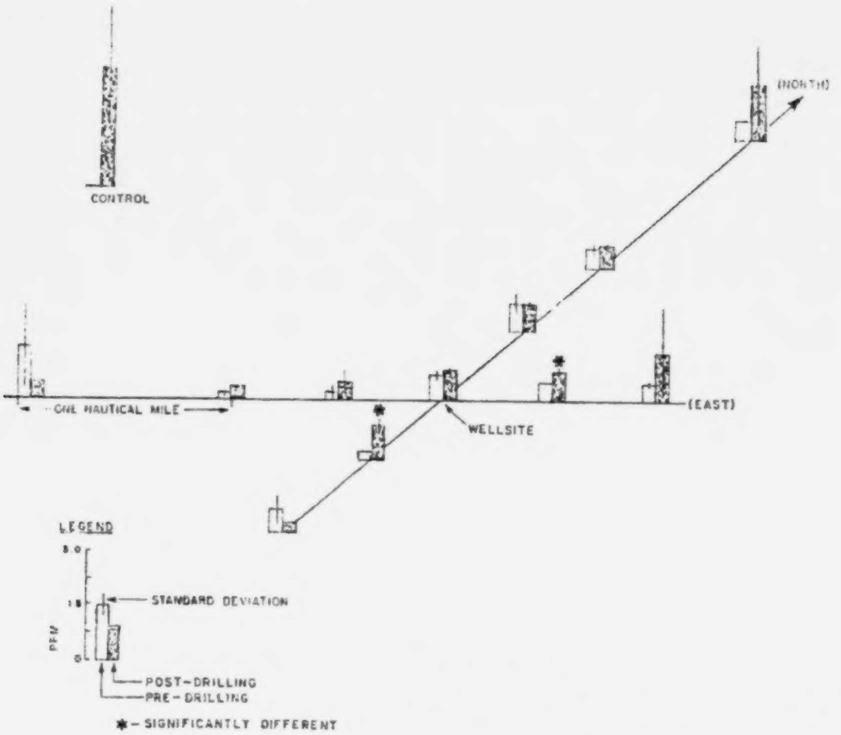

FIGURE 3 WEAK ACID-LEACHABLE BARIUM IN SURFICIAL SEDIMENTS AT OLYMPIA A-12

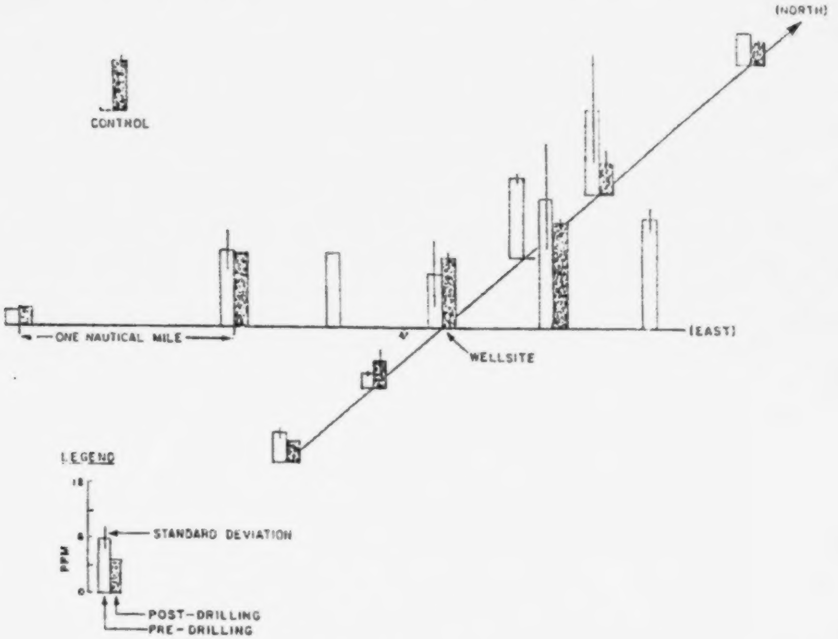

FIGURE 4 TOTAL ZINC IN SURFICIAL SEDIMENTS AT OLYMPIA A-12

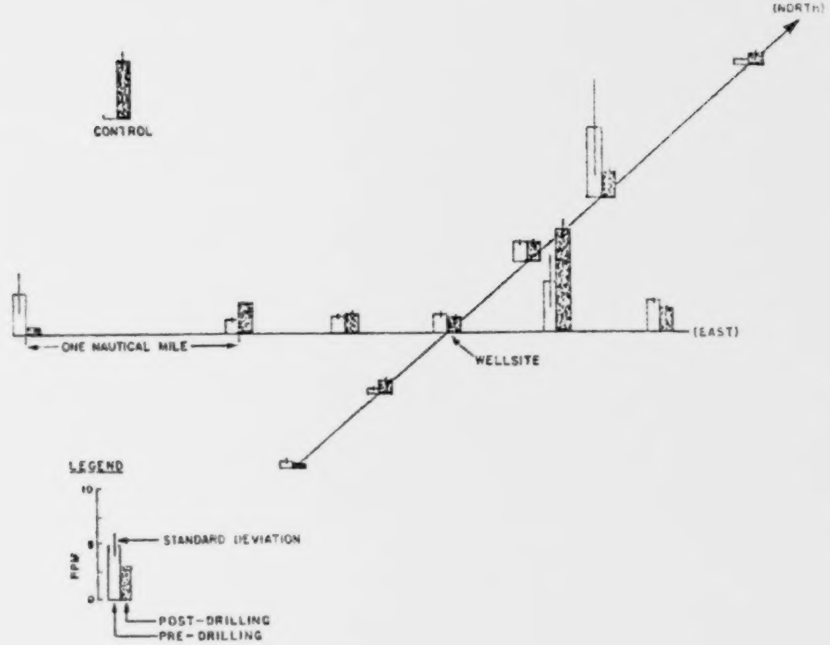

FIGURE 5 WEAK ACID-LEACHABLE ZINC IN SURFICIAL SEDIMENTS AT OLYMPIA A-12

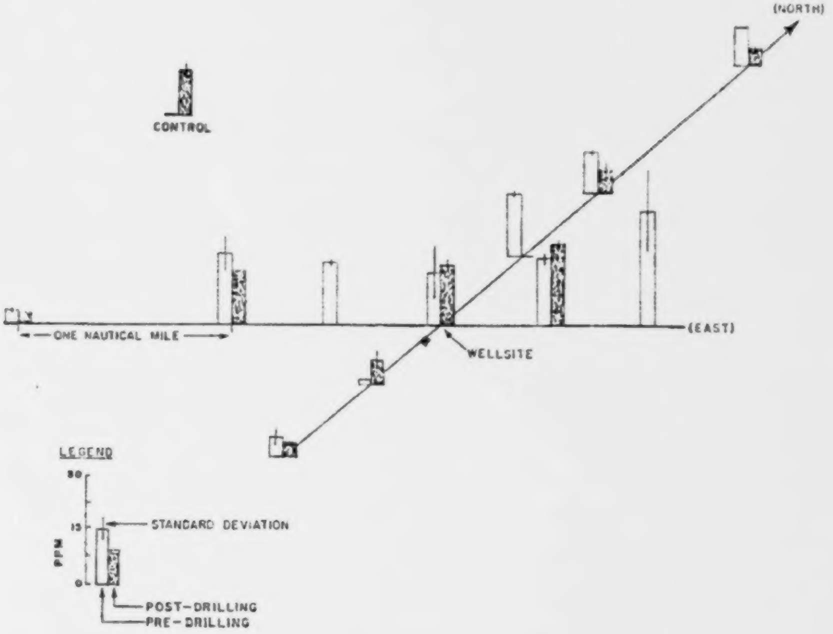

FIGURE 6 TOTAL CHROMIUM IN SURFICIAL SEDIMENTS AT OLYMPIA A-12

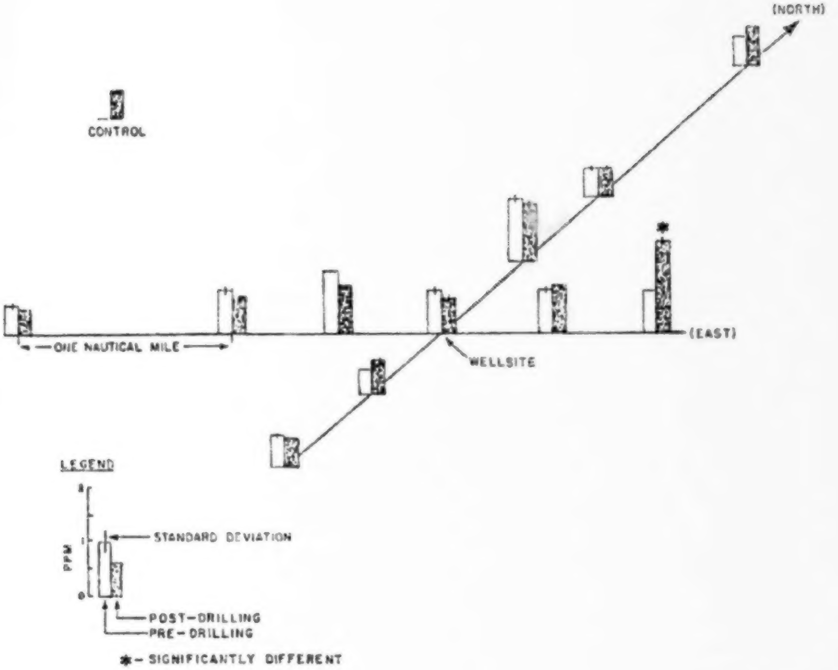

FIGURE 7 WEAK ACID-LEACHABLE CHROMIUM IN SURFICIAL SEDIMENTS AT OLYMPIA A-12

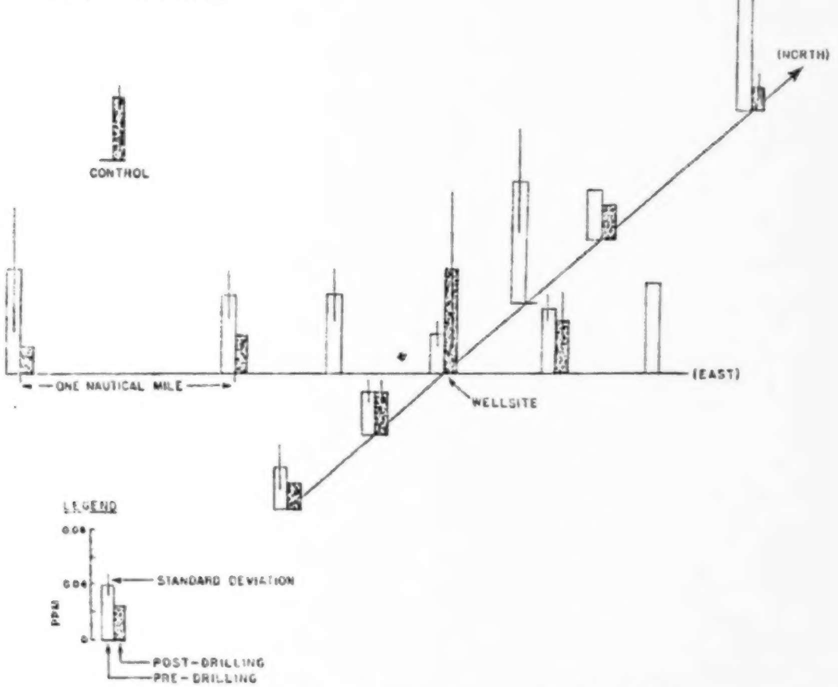

FIGURE 8 TOTAL CADMIUM IN SURFICIAL SEDIMENTS AT OLYMPIA A-12

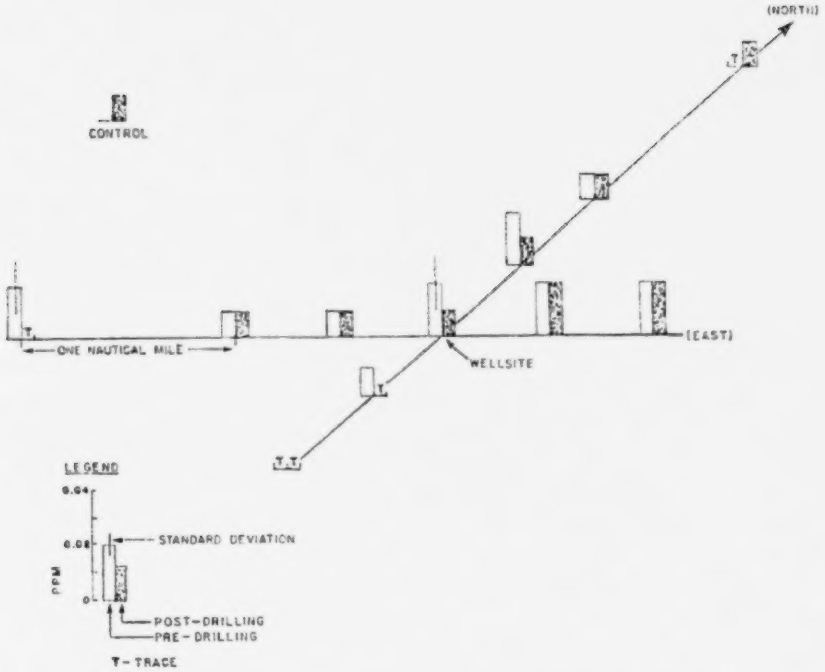

FIGURE 9 WEAK ACID-LEACHABLE CADMIUM IN SURFICIAL SEDIMENTS AT OLYMPIA A-12

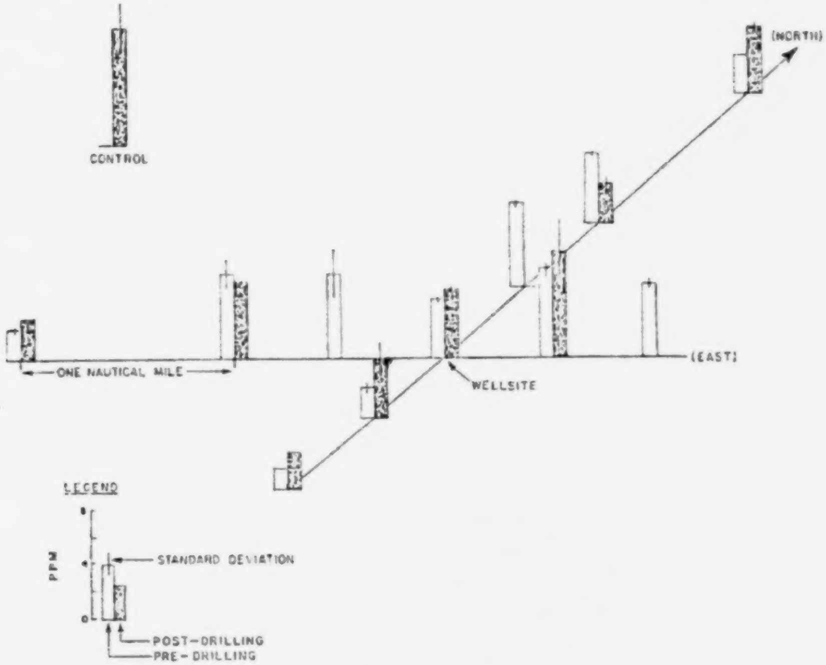

FIGURE 10 TOTAL LEAD IN SURFICIAL SEDIMENTS AT OLYMPIA A-12

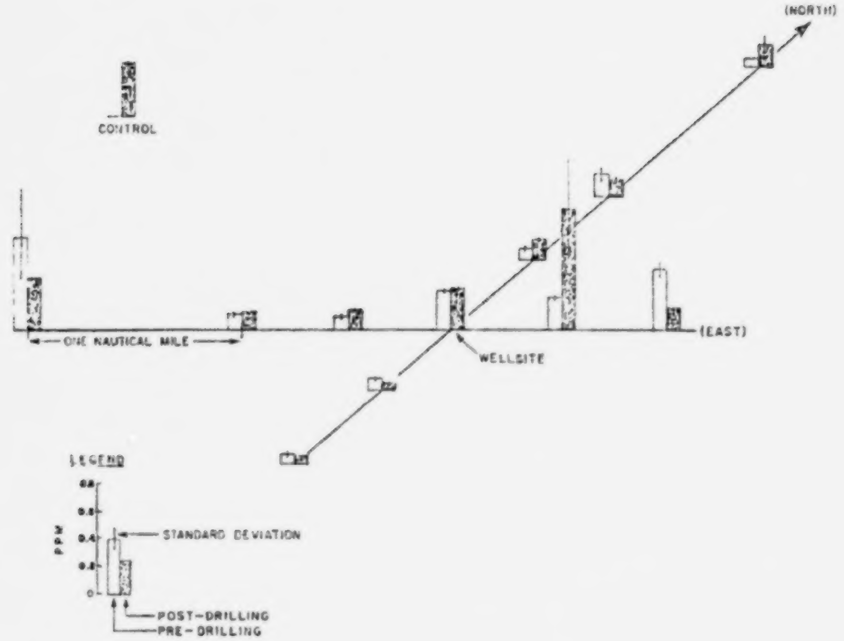

FIGURE 11 WEAK ACID-LEACHABLE LEAD IN SURFICIAL SEDIMENTS AT OLYMPIA A-12

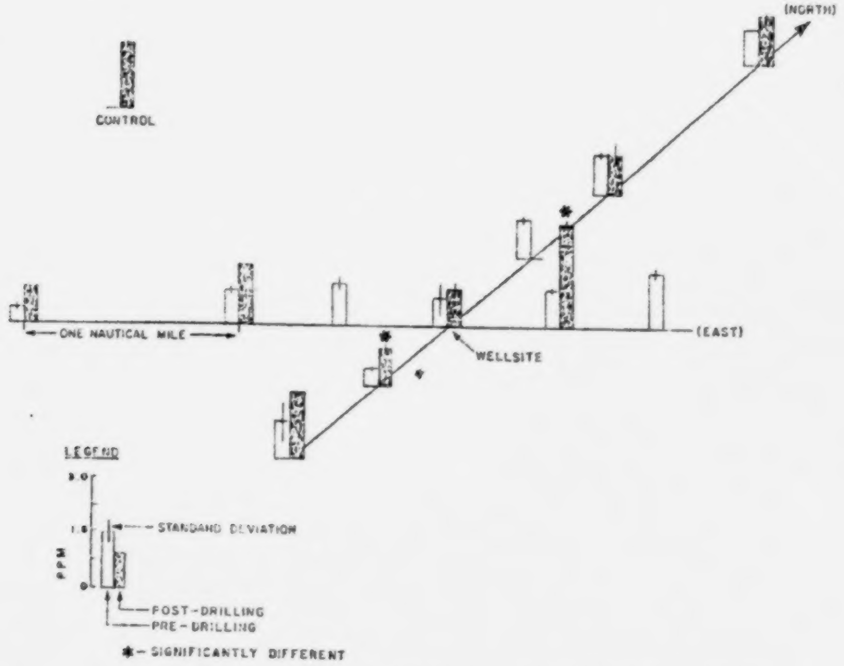

FIGURE 12 TOTAL COPPER IN SURFICIAL SEDIMENTS AT OLYMPIA A-12

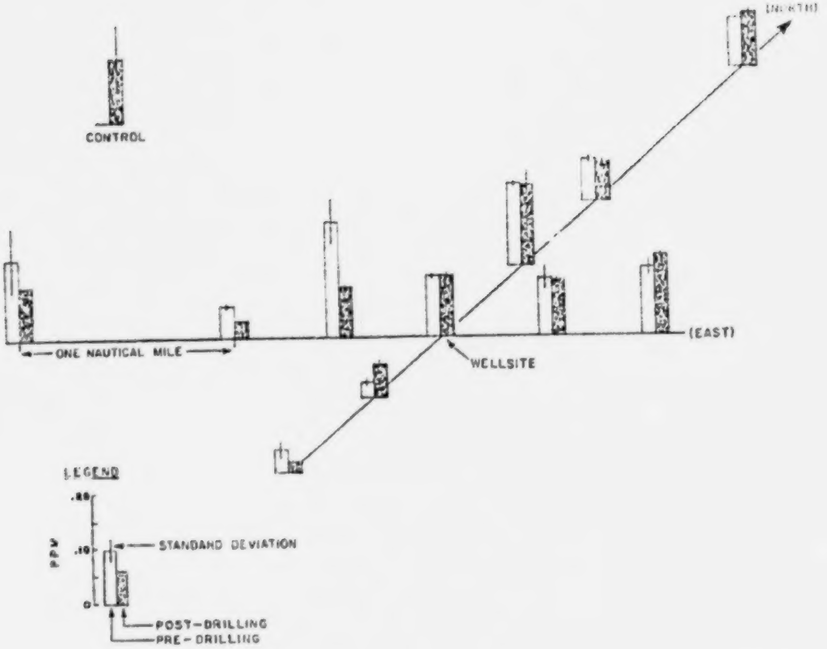

FIGURE 13 WEAK ACID-LEACHABLE COPPER IN SURFICIAL SEDIMENTS AT OLYMPIA A-12

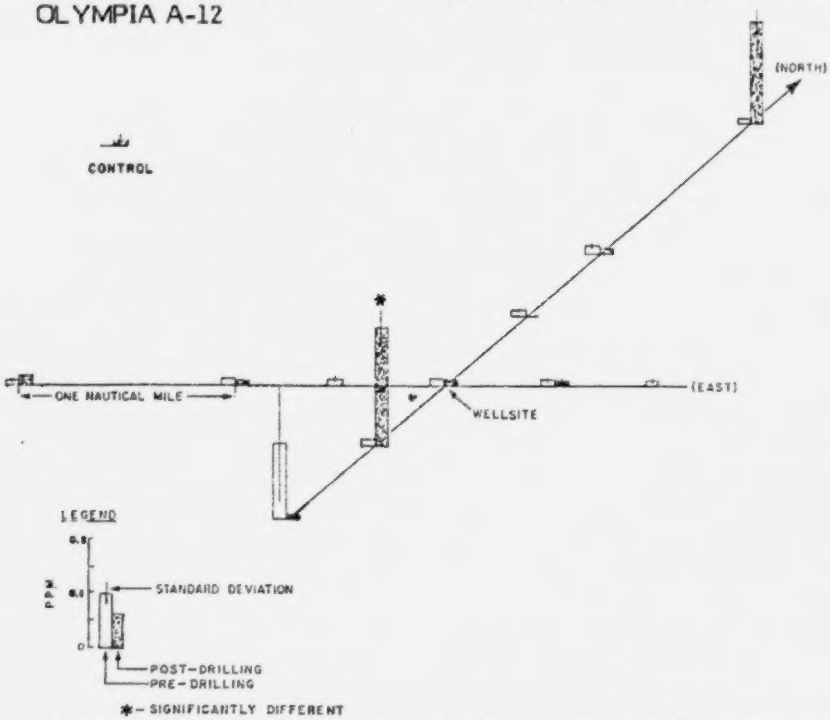

FIGURE 14 TOTAL MERCURY IN SURFICIAL SEDIMENTS AT OLYMPIA A-12

Station 11: Apparent accumulation of weak acid-leachable chromium in post-drilling sediments.

There were suggestions of post-drilling accumulations of barium at other stations, but relatively high variability at these stations precluded statistical significance. The apparent accumulations of barium occurred at stations which had relatively low (and perhaps anomalous) levels in pre-drilling sediments. In other words, although accumulation in post-drilling sediments was indicated, the post-drilling barium concentrations at these stations were not very different from barium levels in pre- and post-drilling sediments at other stations (Figs. 2 and 3). This emphasizes that metal levels in both pre- and post-drilling sediments were patchy within the survey area.

The significant accumulations of chromium, copper, and mercury were generally much more distinct than those of barium, reflecting high post-drilling concentrations exceeding both pre- and post-drilling concentrations in sediments at other stations and at the control (Figs. 7, 12, 14). Although significance could not be tested because of only one pre-drilling sample, there was apparent accumulation of mercury in post-drilling sediments at Station 4.

## Scallop Tissue

Chromium, zinc, and cadmium were analyzed in moderate-size scallops at all stations where scallops occurred. Barium, copper, lead, and mercury were analyzed only in moderate-size scallops from Station 4. Table 5 shows the ranges of metal concentrations in scallop tissue from the Olympia A-12 well site. With the exception of zinc, metal levels in the viscera were consistently higher than those in the adductor muscle. Zinc and barium levels were relatively high in adductor muscle compared to low levels of cadmium, chromium, lead, copper, and mercury. In the viscera, cadmium, barium, and zinc concentrations were high compared to low levels of chromium, copper, lead, and mercury.

TABLE 5 RANGES OF MEAN CONCENTRATIONS (ppm dry weight) OF TRACE METALS IN SCALLOP TISSUE (post-drilling) AT THE OLYMPIA A-12 WELL SITE (only scallops with adductor muscle dry weight between 1.6 and 5.0 g)

| Metal | Adductor Muscle | Viscera |
|---|---|---|
| Chromium[1] | 0.17 - 1.05 | 0.6 - 1.4 |
| Zinc[1] | 30.6 - 77.1 | 29.8 - 82.1 |
| Cadmium[1] | 1.1 - 3.9 | 68.3 - 195.0 |
| Barium[2] | 11.6 ± 17.2 | 85.3 ± 77.3 |
| Copper[2] | 0.12 ± 0.07 | 1.05 ± 0.61 |
| Lead[2] | 0.13 ± 0.25 | 1.13 ± 0.59 |
| Mercury[2] | 0.07 ± 0.03 | 0.14 ± 0.16 |

[1] All stations

[2] Only Station 4 (mean ± standard deviation; n=14)

Figs. 15 - 20 show the mean concentrations of chromium, zinc, and cadmium in scallop adductor muscle and viscera from the Olympia A-12 well site. Unfortunately, there was inadequate scallop tissue from the pre-drilling survey for temporal comparisons. However, comparisons between stations in the post-drilling survey were made and several significantly different metal concentrations were noted. These are summarized below:

| | |
|---|---|
| Station 1: | High level of chromium in the viscera; high level of zinc in the adductor muscle and viscera. |
| Station 4: | High level of chromium in the adductor muscle; high level of zinc in the viscera; low level of cadmium in the viscera. |
| Station 5: | High level of chromium in the viscera. |

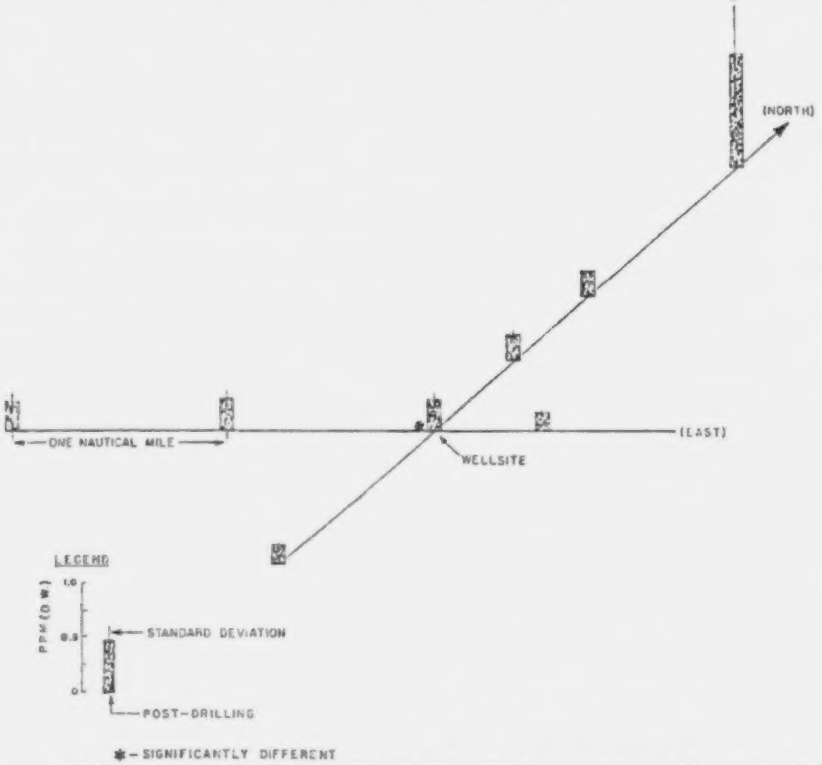

FIGURE 15 CHROMIUM IN SCALLOP ADDUCTOR MUSCLE AT OLYMPIA A-12 (only scallops with adductor muscle dry weight between 1.6 and 5.0 g)

Table 6 shows the whole-animal (soft tissue) concentrations of chromium, zinc, and cadmium in scallops from the Olympia A-12 well site. These concentrations generally reflected the trends in visceral metal concentrations shown in Figs. 16, 18 and 20.

Average ratios of metal concentration in adductor muscle to visceral metal concentration are noted in Table 7. The ratios for individual metals were relatively consistent between stations. However, scallops from Stations 3 and 4 had unusual ratios of adductor muscle metal concentration of visceral metal concentration, compared to those for the other stations.

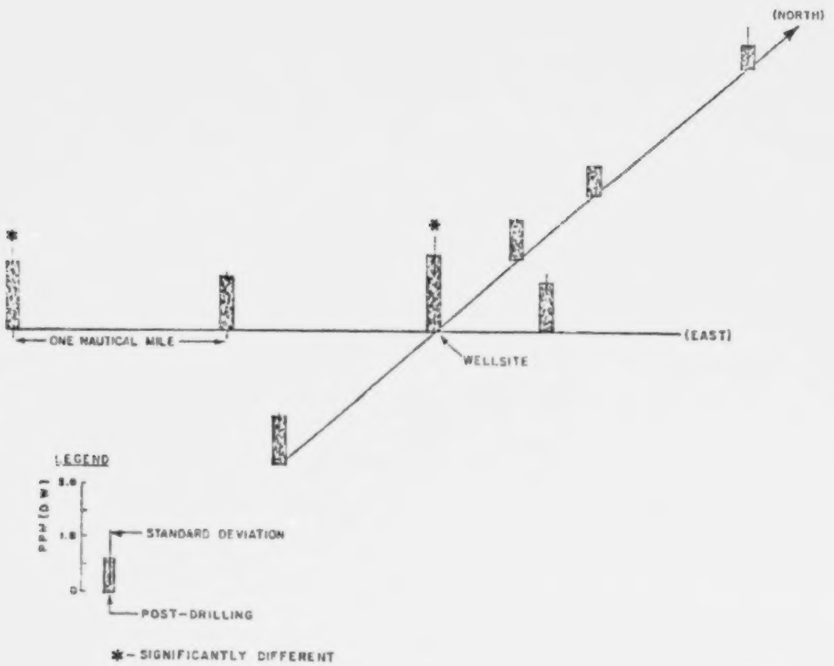

FIGURE 16 CHROMIUM IN SCALLOP VISCERA AT OLYMPIA A-12 (only scallops with adductor muscle dry weight between 1.6 and 5.0 g)

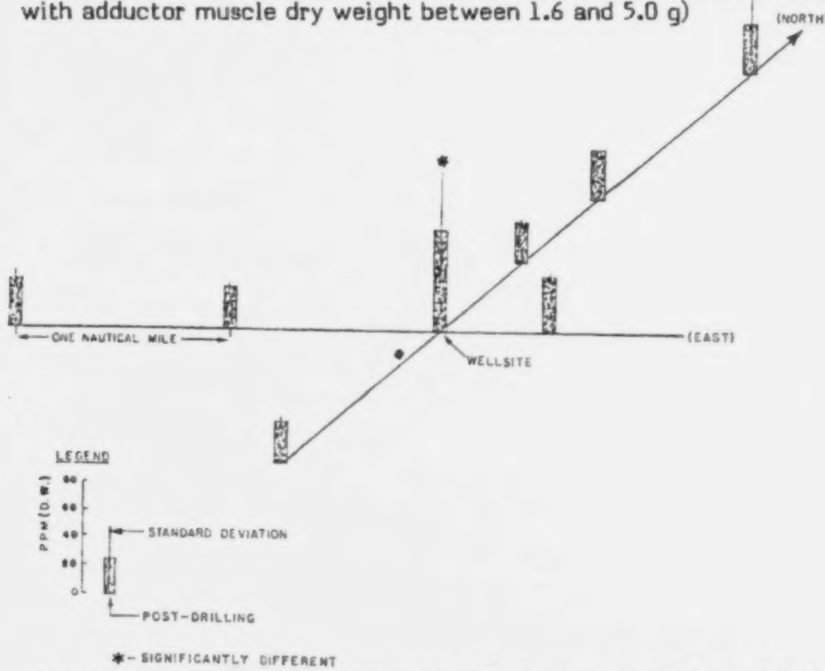

FIGURE 17 ZINC IN SCALLOP ADDUCTOR MUSCLE AT OLYMPIA A-12 (only scallops with adductor muscle dry weight 1.6 and 5.0 g)

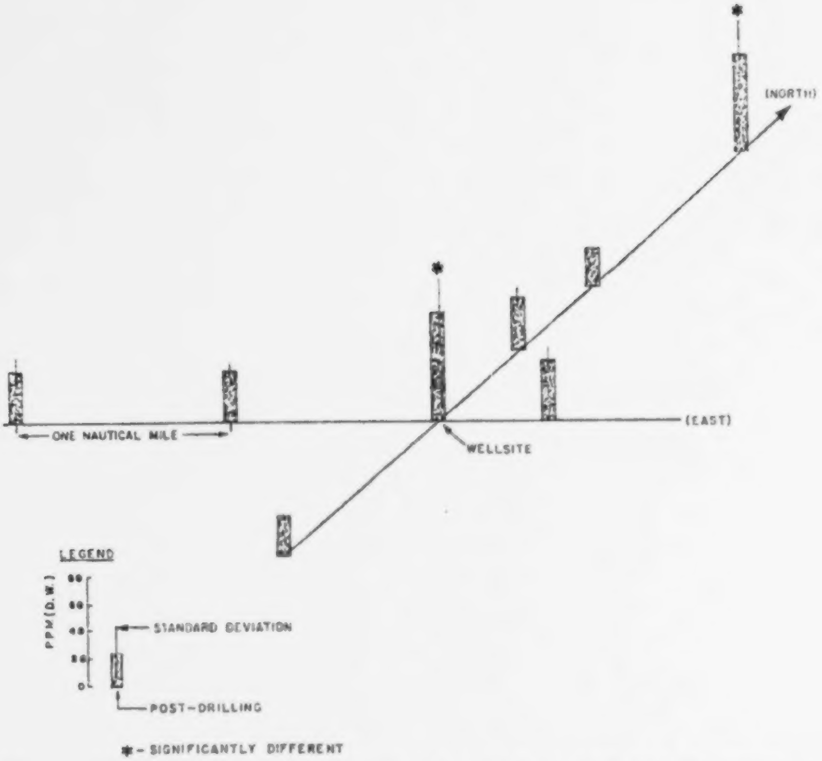

FIGURE 18 ZINC IN SCALLOP VISCERA AT OLYMPIA A-12 (only scallops with adductor muscle dry weight beteen 1.6 and 5.0 g)

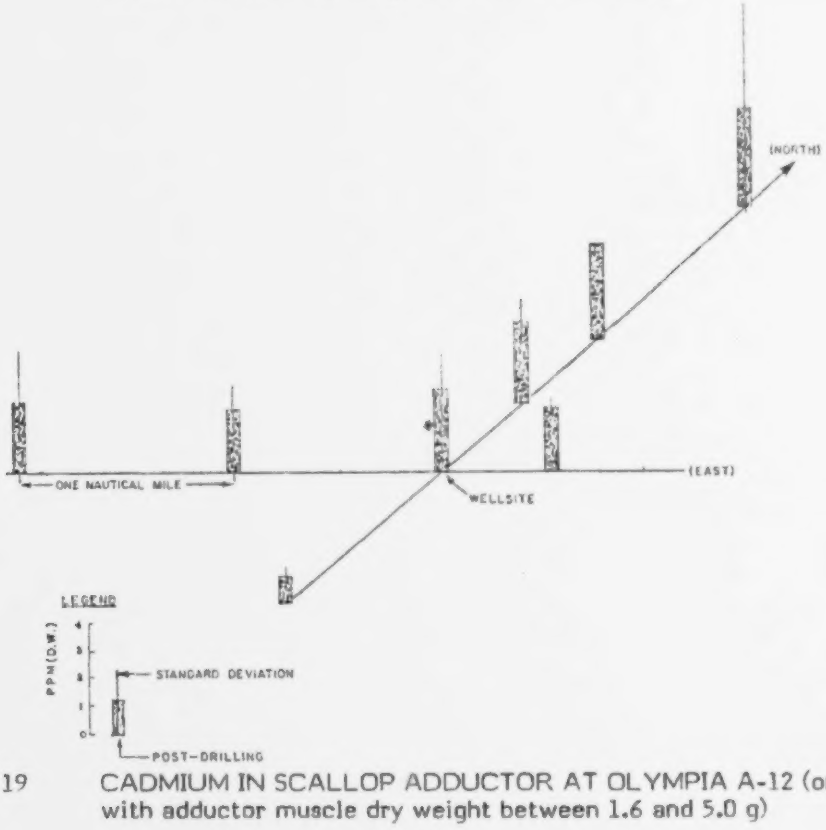

FIGURE 19 CADMIUM IN SCALLOP ADDUCTOR AT OLYMPIA A-12 (only scallops with adductor muscle dry weight between 1.6 and 5.0 g)

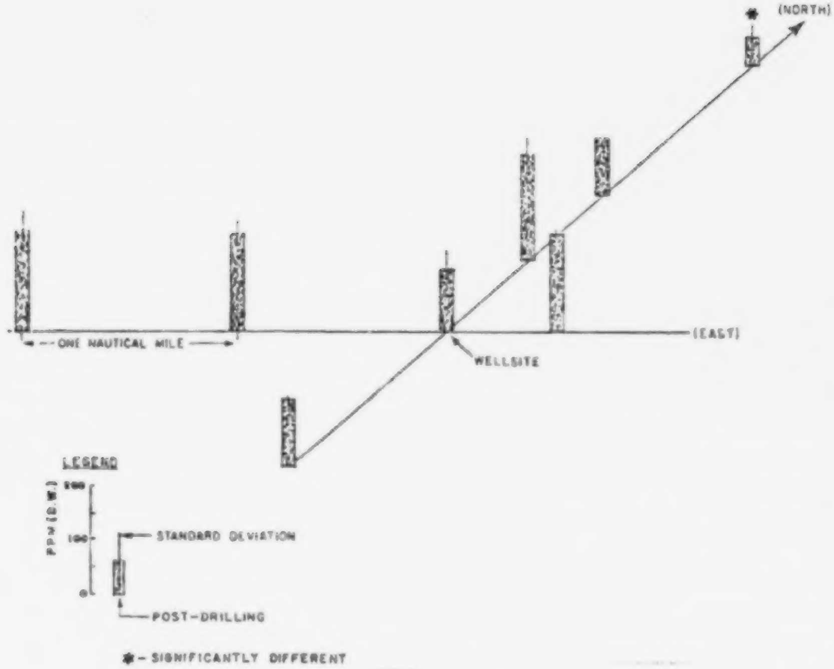

FIGURE 20 CADMIUM IN SCALLOP VISCERA AT OLYMPIA A-12 (only with adductor muscle dry weight between 1.6 and 5.0)

There was no correlation between metal concentrations in scallop tissue and those in the sediments.

## DISCUSSION

### Sediments

The low silt and clay content of surficial sediments at the Olympia A-12 well site, both before and after drilling, reflected the dynamic physical environment in the vicinity of Sable Island. Strong tidal currents and vertical mixing near the Island (Mobil Oil Canada Ltd., 1983) are not conducive to prolonged deposition of fine material associated with drillingwaste. The initial wide dispersion of fine material, such as bentonite clays,

TABLE 6 AVERAGE WHOLE-ANIMAL CONCENTRATIONS (ppm dry weight) OF CHROMIUM, ZINC, AND CADMIUM IN SEA SCALLOPS FROM THE OLYMPIA A-12 WELL SITE

| Station | Chromium | Zinc | Cadmium |
|---|---|---|---|
| 1 | 1.11 | 80.72 | 83.99 |
| 2 | 0.56 | 39.82 | 113.23 |
| 3 | 0.47 | 35.61 | 50.72 |
| 4 | 0.81 | 56.24 | 35.13 |
| 5 | 1.01 | 38.27 | 146.32 |
| 6 | 0.78 | 37.47 | 123.93 |
| 8 | 0.71 | 31.90 | 108.95 |
| 10 | 0.63 | 44.47 | 115.29 |

TABLE 7 AVERAGE RATIOS OF ADDUCTOR MUSCLE CONCENTRATION TO VISCERAL CONCENTRATION FOR CHROMIUM, ZINC, AND CADMIUM IN SEA SCALLOPS FROM THE OLYMPIA A-12 WELL SITE

| Station | Chromium | Zinc | Cadmium |
|---|---|---|---|
| 1 | 0.197 | 0.938 | 0.028 |
| 2 | 0.300 | 0.798 | 0.016 |
| 3 | 0.400 | 1.350 | 0.032 |
| 4 | 1.875 | 0.636 | 0.057 |
| 5 | 0.213 | 0.991 | 0.016 |
| 6 | 0.272 | 0.828 | 0.013 |
| 8 | 0.193 | 0.947 | 0.008 |
| 10 | 0.207 | 0.974 | 0.014 |

during discharge into the water and subsequent reworking on the bottom would have reduced the potential for localized accumulations of drilling waste in surficial sediments.

The relatively coarse sediments near Sable Island were expected to have low concentrations of trace metals, as particle size is a controlling factor in the abundance of total metals, with detrital host minerals being mostly fine grained (Loring, 1979). This was the case for both pre- and post-drilling sediment samples. In fact, the trace metal concentrations at Olympia A-12 (with the possible exception of mercury) were considerably lower than levels recorded by Loring (1979) in texturally-equivalent Bay of Fundy sediments. This indicates that surficial sediments near Sable Island, as expected, have not been subjected to chronic anthropogenic metal inputs, or, at least, metals have not been able to settle and accumulate there in the long term.

In view of the dynamic physical regime at the Olympia A-12 well site, large accumulations of trace metals in surficial sediments were not expected. However, there was apparent accumulation of barium, copper, and mercury at stations within 0.5 nautical miles south and east of the well site, but not at the well site itself. These metals are potentially associated with barite (Crippen et al., 1980; Neff, 1981), large quantities of which were discharged during drilling of Olympia A-12. Because of its high density, barite initially drops out very near the rig (EG and G Environmental Consultants, 1982), although strong near-bottom currents may considerably rework this barite and cause an apparent reduction in barite contaminant levels through lateral transport and entrainment in sandy sediments (Houghton et al., 1980). The accumulation of barium, copper, and mercury south and east of the well site is consistent with dispersal of the original barite discharge in the direction of the prevailing waves and residual current. The dispersing forces appear to have been strong enough to limit metal accumulations to only 2 - 3 x the pre-drilling levels. However, the post-drilling accumulations of mercury at two stations were much higher than this (about 20 x pre-drilling levels) and suggest that post-discharge behaviour of mercury differs from that of barium and copper.

Chromium is a prominent component of chrome lignite, a drilling mud additive. Discharged chromium, associated with organic or clay particles, unlike barite, is subject initially to wide dispersal in the water column (Trocine and Trefry, 1983). Detection of chromium accumulation in surficial sediments near Olympia A-12 was therefore not expected. In fact, the only detectable accumulation was that of weak acid-leachable chromium one nautical mile east of the well site, downcurrent from the original discharge.

We conclude that metal accumulation in surficial sediments soon after drilling, related to discharge of drilling-waste at Olympia A-12, was spatially limited and, with the exception of mercury, limited to 2 - 3 fold increases over pre-drilling levels.

### Scallop Tissue

Differences in sample handling, analytical techniques, and size of animals make comparisons of scallop trace metal data from various studies difficult and perhaps meaningless. We therefore limit our discussion of the scallop tissue data in this study (with one exception) to inferences about metal accumulation at the Olympia A-12 well site. This in itself is limited because of a lack of scallop tissue from the pre-drilling survey and from the control site. In the absence of temporal controls, we have assumed that spatial differences in tissue metal levels within the post-drilling survey area may have reflected inputs from drilling-waste or other rig-associated features.

With the exception of zinc, all metals, especially cadmium, showed a higher concentration in the viscera compared to the adductor muscle. This indicates limited sequestering of metals in the adductor muscle. The higher levels of metals in the scallop viscera may have reflected high metal levels normally found in the kidney (Carmichael et al., 1980), digestive gland, and other organs include in the viscera. The relatively even distribution of zinc in scallop adductor muscle and viscera has been observed in other molluscs (Carmichael et al., 1980) and may be related to zinc being an essential trace element and a constituent of many enzymes (Scrutton, 1973).

Studies of benthic fauna near well sites have demonstrated uptake of barium and subsequent depuration after cessation of drilling, limited uptake of chromium (EG and G Environmental Consultants, 1982), and uptake of mercury (Crippen et al., 1980). Liss et al., (1980) showed that the kidneys of sea scallops exposed to drilling muds accumulated barium and chromium. The adductor muscle showed only a very slight increase in barium during exposure.

Barium was not analyzed in scallops from all stations. However, results from Station 4, 2 nautical miles north of the well site, suggest accumulation of barium in scallop tissue when compared to data reported by Liss et al., (1980). They recorded barium concentrations of 100 ppm (dry weight) in scallop kidneys exposed to synthetic drilling muds and barium levels in exposed adductor muscle ranging between 8 and 12 ppm. Barium levels in control kidneys and adductor muscles were less than 20 and 5 ppm, respectively (Liss et al., 1980). The mean barium concentrations in viscera (including metal-laden kidney) and adductor muscle from moderate-size scallops at Station 4 near Olympia A-12 were 85 and 11 ppm, respectively. The suggestion of barium accumulation in scallops at Station 4 remains tenuous because of possible differences in methodology and scallop size between the two studies. Additional barium analyses of scallops from other stations near Olympia A-12 would have been useful.

Chromium, zinc, and cadmium were analyzed in all the scallops collected. There was apparent accumulation of chromium at the well site, and two nautical miles north and west of the well site. The probable origin of the chromium was the chrome lignite discharged during drilling. Because the chromium in this discharge usually adheres to clay-sized particles which are initially widely dispersed, we expected tissue accumulation to occur at some distance from the well site where the particles might have settled out, rather than near the well site itself. However, we also expected tissue accumulation to occur downwind and downcurrent from the rig; that is, south and east of the well site. The spatial pattern of scallop tissue accumulation of chromium is not easily explained.

Zinc accumulation in scallop tissue was evident at the well site and two nautical miles north of the well site. There were at least two possible sources of this apparent zinc contamination. Zinc is present in both barite and bentonite clay (Crippen et al., 1980; Neff, 1981) and may have accounted for contamination at both the well site and north of the well site. Zinc from sacrificial anodes on the rig may also have contributed to scallop tissues accumulation at the well site.

Cadmium accumulation in scallop viscera was interesting. Significantly low concentrations of cadmium in scallop viscera were found at Station 4, where significantly high concentrations of visceral zinc were detected. Cadmium forms protein complexes which are similar to those of zinc, and competitive effects between the two metals have been observed in bivalves (Cooke et al., 1979). Increases in the availability of cadmium appear to decrease the tissue concentrations of zinc. There is some evidence for this inverse relationship at Olympia A-12, although it is not clear which metal is controlling the relationship.

An examination of the ratios of adductor muscle metal concentration to visceral metal concentration revealed that scallops from Stations 3 and 4 were somewhat different from those at other stations. The unusual ratios may have been due to post-mortem migration of metals between tissues, or may have been related to the relatively low visceral weights (post-spawning or stress-?) recorded at these stations. Regardless of the ratios noted in Table 7, the whole-animal metal concentrations still reflected the trends in visceral metal concentrations because of the relatively large metal burden (at least for chromium and cadmium) in viscera compared to the adductor muscle.

The lack of correlation between metal concentrations in sediments and scallop tissue was expected. Scallops filter near-bottom water and may accumulate metals adsorbed to particles which are settling on the bottom or resuspending. Because they can excrete metals and eventually show a net reduction in tissue metal concentration after cessation of drilling-waste discharge (Liss et al., 1980), they may only serve as integrators of recent drilling-waste movements near the bottom. When we examined scallops two weeks after cessation of drilling, we may have documented only the recent discharge history, rather than the ultimate fate of discharge from the whole drilling program. On the other hand, the sediments, without very careful sampling of the topmost (and perhaps ephemeral) layer, would serve to document the longer term fate of metals from drilling-waste; i.e., those metals that have been entrained in the top few centimetres of sediment. Thus, scallop tissue contamination was indicated at the well site, and north and west of the well site, whereas sediment contamination was evident south and east of the well site. a haze of drilling-waste discharge occurring northwest of the rig towards the end of the drilling program, and filtered by scallops, might eventually have contributed to sediment contamination downcurrent from the well site.

We conclude that sediment and scallop tissue accumulation of metals associated with drilling-waste discharge at Olympia A-12 very soon after drilling were both spatially limited and patchy, being dependent on both short term and long term current patterns. Despite evidence of localized sediment accumulations, the concentrations of all metals, except mercury, were still lower than metal concentrations in texturally-equivalent coastal sediments. Barium alone does not appear to clearly indicate the post-discharge movement of drilling-waste. Analysis of chromium and contaminants of barite is recommended as well.

## ACKNOWLEDGEMENTS

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# CONTINUOUS-FLOW DEVICES FOR EXPOSING MARINE ORGANISMS TO THE WATER-SOLUBLE FRACTION OF CRUDE OIL AND ITS COMPONENTS

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MOLES, A., S.D. RICE, and S. ANDREWS. 1985. Continuous-flow devices for exposing marine organisms to the water-soluble fraction of crude oil and its components. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 53-61.

The devices produce stable concentrations of aromatic hydrocarbons that can be used in continuous-flow toxicity tests. The crude-oil mixing device produces a stable (>5% deviation) water-soluble fraction of 2.5 mg/l total aromatic hydrocarbons for 30-40 days. The device uses a gentle flow of water to dissolve aromatic components in a layer of crude oil floating on a column a 2-m column of seawater. Because the water does not pick up oil droplets as it passes through the column, a water-soluble fraction is produced rather than a dispersion. The other device, a syringe pump, introduces compounds directly into a water stream and produces a stable (<1% deviation for toluene) solution of monoaromatic hydrocarbons of any desired concentration or mixture up to the maximum solubility of the compounds. Both devices give reproducible results, are inexpensive, easily maintained, safe, and adaptable to many toxicants.

MOLES, A., S.D. RICE, and S. ANDREWS. 1985. Continuous-flow devices for exposing marine organisms to the water-soluble fraction of crude oil and its components. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 53-61.

Ces dispositifs produisent des concentrations stables d'hydrocarbures aromatiques qu'on peut utiliser dans des tests de toxicité à flux continu. Le dispositif de mélange de pétrole brut produit une fraction soluble dans l'eau stable (écart inférieur à 5 %) de 2,5 mg/L d'hydrocarbures aromatiques totaux, pendant 30 à 40 jours. Le dispositif utilise un courant modéré d'eau pour dissoudre les composants aromatiques dans une couche de pétrole brut flottant sur une colonne de 2 m d'eau de mer. Du fait que l'eau ne rassemble pas les gouttelettes de pétrole lorsqu'elle passe à travers la colonne, il se produit une fraction soluble dans l'eau au lieu d'une dispersion. L'autre dispositif, une pompe à seringue, introduit les produits directement dans un courant d'eau et produit une solution stable (écart inférieur à 1 % pour le toluène d'hydrocarbures monoaromatiques de toute concentration ou composition désirée jusqu'à la solubilité maximale des composants. Les deux dispositifs donnent des résultats reproductibles, sont économiques, d'un entretien facile, sûrs et s'adaptent à de nombreux produits toxiques.

toluene solution over 20 days at 260 nm, and the variance was <1% of the concentration in the range from 20 to 60 mg/l.

A major advantage of the syringe-pump device is that a water-soluble fraction of crude oil can be simulated by mixing known concentrations of aromatic hydrocarbons, a valuable application in the studying synergistic interactions of aromatic hydrocarbons. Aromatic hydrocarbons can be mixed in the syringe in the same ratios as they appear in crude oil, or in any desired ratio. The use of syringe pumps to introduce compounds into water is not new, but the use of syringe pumps to introduce organic compounds and their mixtures is.

## ACKNOWLEDGEMENTS

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## FOOTNOTES

[1] Present address: 9005 Gee Street, Juneau, AK 99802.

[2] Reference to trade names does not imply endorsement by the National Marine Fisheries Services, NOAA.

## FIGURE LEGENDS

Figure 1 Device for making water-soluble fractions of crude oil.

Figure 2 Reliability of the oil-mixing device at 10°C, as measured by total concentration (mg/l) of 10 aromatic hydrocarbons (see text) in the undiluted seawater-soluble fraction of Cook Inlet crude oil. Aromatic hydrocarbons in the effluent from the oil-mixing device were measured daily for 40 days by gas chromatography. The 95% confidence limits are shown.

Figure 3 The syringe-pump device for dissolving aromatic hydrocarbons in seawater.

données limitées sur l'accumulation et l'élimination du cadmium, on a procédé à la détermination des niveaux tissulaires du cadmium dans le foie, le rein et le muscle. De jeunes truites arc-en-ciel (Salmo gairdneri) ont été exposées à un flot continu d'eau saumâtre contenant 0, 10 et 100 microgrammes de cadmium par litre pendant 30 semaines suivies d'une période de rétablissement de 57 semaines. Les échantillons ont été pris après 18, 30, 55 et 87 semaines. Une importante accumulation de cadmium, selon la dose, a été trouvée dans le foie, après 18 et 30 semaines d'exposition, tandis que l'accumulation dans le muscle était moins prononcée. Dans le foie et le rein, l'élimination du cadmium s'est montrée très lente, semblant indiquer une demi-vie biologique du cadmium dans ces tissus d'une durée de plus de un an. Par ailleurs, dans des poissons examinés après 87 semaines, on a trouvé une forte corrélation (r = 0,90) entre les niveaux tissulaires du cadmium dans le foie et dans le rein. Une anémie s'est révélée par une baisse de l'hématocrite en cours d'exposition, mais ne s'est pas présentée après rétablissement dans l'eau sans cadmium. Une hypocalcémie et une hypermagnésémie ont été observées pendant l'exposition, mais ne se sont pas manifestées après rétablissement. Une hyperglycémie persistante a été notée pendant l'exposition et le période de rétablissement, tandis qu'on notait une baisse du glycogène musculaire après 55 semaines. D'une façon générale, les modifications observées correspondent aux effets du cadmium sur le poisson précédemment rapportés. Cependant, les résultats de cette étude semblent indiquer que la truite arc-en-ciel pourrait se rétablir des perturbations de la régulation ionique et de son hématologie, tandis que l'influence marquée du cadmium sur le métabolisme des hydrates de carbone persiste et risque d'être dommageable pour les processus physiologiques qui dépendent de l'intégrité de l'homéostase du glucose.

# MARINE MONITORING PROGRAM, NANISIVIK, N.W.T. - CASE HISTORY, PROBLEMS, AND NEEDS FOR FUTURE RESEARCH

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METIKOSH, S. 1985. Marine monitoring program, Nanisivik, N.W.T.- case history, problems and needs for future research. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 65-66.

Nanisivik is a lead and zinc mine located on the Borden Pennisula on Baffin Island, and is the first such operation in Arctic Canada. Tailings from the milling process are pumped to a containment area where they are treated by gravity sedimentation. The supernatant is discharged into a freshwater creek which empties into Strathcona Sound approximately six kilometers downstream. Effluent quality standards specify the minimum acceptable levels of heavy metals that can be discharged to the receiving waters.

The need to assess the impacts of heavy metals on the marine environment was recognized by regulatory agencies of the Federal Government and a program to monitor yearly changes in trace metal concentration in sediments and biota of Strathcona Sound was established. The results of the first post operational phase were recently published in Fisheries and Aquatic Sciences Technical Report No 1082 (Fallis 1982).

Subsequent studies were conducted in an effort to assist in assessing the significance of these results. Studies included additional trace metal body burden determination, histopathological and biochemical determinations of stress in the mollusc *Mya truncata*, fresh and marine water quality analyses and geochemical analyses of superficial marine sediment.

Results from these investigations will be presented in conjunction with a discussion of difficulties encountered in developing a linkage between the results obtained and adverse effects on the marine environment. Areas where additional research is required will also be discussed.

METIKOSH, S. 1985. Marine monitoring program, Nanisivik, N.W.T.- case history, problems and needs for future research. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 65-66.

Nanisivik est une mine de plomb et de zinc située sur la péninsule Borden de l'île Baffin, la première à fonctionner dans l'Arctique canadien. Les rejets du processus d'extraction sont pompés dans une zone réservoir où ils sont traités par sédimentation par gravité. La partie surnageante est déversée dans une baie d'eau douce qui se vide dans la baie Strathcona, à environ six kilomètres en aval. Les normes de qualité de l'effluent

précisent les niveaux maximaux acceptables de métaux lourds qui peuvent être déversés dans les eaux réceptrices.

Les organismes de réglementation du gouvernement fédéral ont reconnu la nécessité d'évaluer l'effet des métaux lourds sur l'environnement marin, et on a établi un programme de surveillance des modifications annuelles de concentrations d'oligo-éléments dans les sédiments et le boite de la baie Strathcona. Les résultats de la première phase post-opérationnelle ont été récemment publiés dans le Rapport technique sur les sciences halieutiques et aquatiques n° 1082 (Fallis 1982).

Par la suite, des études ont été menées dans le but d'aider à évaluer l'importance de ces résultats. Parmi ces études figure la détermination de la charge corporelle additionnelle en métaux à l'état de trace, les déterminations histopathologiques et biochimiques du stress chez le mollusque Mya truncata, des analyses de qualité de l'eau douce et de l'eau salée, ainsi que des analyses géochimiques du sédiment marin superficiel.

Les résultats de ces recherches seront présentés en association avec une analyse des difficultés rencontrées dans l'établissement d'un lien entre les résultats obtenus et les effets négatifs sur l'environnement marin. On procédera également à l'examen des domaines nécessitant une recherche complémentaire.

# ORGANOCHLORINE COMPOUNDS AND HEAVY METALS IN POLAR BEARS FROM THE WESTERN CANADIAN ARCTIC, 1982

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NORSTROM, R.J. and R.E. SCHWEINSBURG. 1985. Organochlorine compounds and heavy metals in polar bears from the Western Canadian Arctic, 1982. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 67-69.

The diet of the polar bear is almost exclusively seals, making it an ideal species to detect contaminants which may be present in arctic marine food chains and tend to bioconcentrate. Analysis of polar bears, seals and fish in the early 1970's showed the presence of PCBs and DDT-related compounds across the whole Canadian Arctic. We have repeated this study in the Western Arctic from Tuktoyaktuk to Barrow Straight, and included metals in the survey. Sixty-seven liver and fat samples were taken by Inuit during the regular hunting season in 1981-82. Individual liver samples were analyzed for organochlorine compounds by GC/ECD and GC/MS; for Ca, Cu, Fe, K, Mg, Mn, P, Zn, Ag, As, Se, Sr, and V by ICAP and for Hg and Cd by AA. Fat samples were pooled on an equal weight basis from six geographical areas, and analyzed for organochlorines.

PCBs were the predominant residue in fat, at levels ranging from 2.6-5.0 mg/kg lipid. These levels are similar to those found in the early 1970's. Greater than 80% of the PCBs were accounted for by 5 congeners: one penta-, two hexa-and two hepta-CBs. The second most important group of residues were related to technical chlordane:

Compound "C", 2-chlorochlordene, a chlorochlordene isomer, a nonachlor isomer, an exychlordane isomer, oxychlordane and heptachlor epoxide. Oxychlordane accounted for ca. 60% of the total chlordane residues, which ranged from 1.1 to 2.1 mg/kg lipid. Oxychlordane residues predominated in liver, ranging from 1.1 to 2.0 mg/kg wet weight, twice that of the PCB levels. Other residues identified were penta- and hexachlorobenzene, α-HCH, dieldrin, p,p'-DDE and p,p'-DDD. Total DDT-related residues were about four times lower than those found in the early 1970's. The geographical distribution of organochlorines was relatively even, suggesting that the source was one or both of Ocean currents from the Beaufort Sea which move through the Canadian Arctic archiphelago, or uniform atmospheric deposition. The Arctic Ocean may be contaminated from Atlantic water inflow, riverine sources in the U.S.S.R., or atmospheric transport from a variety of possible sources.

Greater geographical variation was found for heavy metals in liver, particularly mercury and cadmium, probably reflecting the geochemical characteristics of the area represented by the food web of the polar bear subpopulations. Cadmium levels were 0.8 - 0.9 mg/kg wet weight in the Victoria Straight/Barrow Straight area, 0.25 - 0.5 mg/kg elsewhere. Mercury levels were highest in the Beaufort Sea (67 ± 71 mg/kg) and Viscount Melville Sound (93 ± 65 mg/kg), intermediate in Amundsen Gulf (44 ± 35 mg/kg), and lowest in Victoria Straight/Franklin Straight (29 ± 27 mg/kg), Hadley Bay (23 ± 16 mg/kg)

and Barrow Straight (22 ± 11 mg/kg). Mercury levels were highly correlated to those of Se, at a Hg/Se molar ratio of 1.27.

NORSTROM, R.J. and R.E. SCHWEINSBURG. 1985. Organochlorine compounds and heavy metals in polar bears from the Western Canadian Arctic, 1982. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 67-69.

L'ours blanc se nourrissant presque exclusivement de phoque, il constitue l'espèce idéale pour détecter les contaminants qui peuvent être présents dans les chaînes alimentaires marines arctiques et qui ont tendance à se bioconcentrer. L'analyse des ours blancs, des phoques et du poisson, au début des années soixante-dix, a montré la présence de BPC et de composés liés au DDT à travers toute la région arctique du Canada. Nous avons renouvelé cette étude dans la région ouest de l'Arctique, de Tuktoyaktuk jusqu'au détroit de Barrow, en incluant les métaux dans notre enquête. Soixante-sept échantillons de foie et de graisse ont été pris par les Inuit au cours de la saison de chasse régulière en 1981-1982. Des échantillons de foie individuels ont été analysés, à la recherche de composés organochlorés par chromatographie en phase gazeuse et détecteur à capture électronique et par chromatographie en phase gazeuse accouplée à la spectrophotométrie de masse; la recherche de Ca, Cu, Fe, K, Mg, Mn, P, Zn, Ag, As, Se, Sr et V a été faite au moyen de plasma d'argon à couplage inductif; celle de Hg et de Cd par absorption atomique. Les échantillons de graisse ont été regroupés à poids égal à partir de six régions géographiques, et analysés en vue d'y rechercher les organochlorés.

Dans la graisse, les BPC ont été les résidus prédominants, à des niveaux variant entre 2,6 et 5,0 mg/kg de lipides. Ces niveaux étaient similaires à ceux qui avaient été déterminés au début des années soixante-dix. Plus de 80 % des BPC on été représentés par 5 congénères: un penta, deux hexa et deux hepta-BC. Le deuxième groupe de résidus, en importance, était relié au chlordane technique: le composé C, le 2-chlorochlordène, un isomère du chlorochlordène, un isomère nonachloré, un isomère oxychlordane, l'oxychlordane et une époxyde de l'heptachlore. L'oxychlordane a représenté environ 60 % du total des résidus de chlordane, variant de 1,1 à 2,1 mg/kg de lipides. Dans le foie, ce sont les résidus d'oxychlordane qui ont prédominé, variant de 1,1 à 2,0 mg/kg de poids humide, soit deux fois les niveaux de BPC. Les autres résidus identifiés ont été le penta et l'hexachlorobenzène, l'a-HCH, le dieldrine, p,p'-DDE et p,p'-DDD. Le total des résidus liés au DDT a été quatre fois inférieur à celui qui a été relevé au début des années soixante-dix. La distribution géographique des organochlorés a été relativement uniforme, semblant indiquer que la source est l'un des courants océaniques (ou les deux) provenant de la mer de Beaufort, et se déplace à travers l'archipel canadien arctique, ou un dépôt atmosphérique uniforme. Il se peut que l'océan Arctique soit contaminé par les eaux atlantiques, par les sources fluviales de l'U.R.S.S. ou la dissémination atmosphérique à partir de diverses sources possibles.

Une variation géographique plus importante a été notée dans le foie pour les métaux lourds, en particulier le mercure et le cadmium, correspondant probablement aux caractéristiques géochimiques de la région représentée par le réseau alimentaire des sous-populations d'ours blancs. Les niveaux de cadmium variaient entre 0,8 et 0,9 mg/kg de poids humide dans la région du détroit de Victoria/détroit de Barrow, et entre 0,25 et 0,5 mg/kg, dans les autres régions. Les niveaux de mercure ont été les plus élevés dans la mer de Beaufort (67 ± 61 mg/kg) et dans le détroit du Vicomte-Melville (93 ± 65 mg/kg), intermédiaires dans le golfe Amundsen (44 ± 35 mg/kg), et les plus bas dans la région du détroit de Victoria/détroit de Franklin (29 ± 27 mg/kg), la baie Hadley (23 ± 16 mg/kg) et le détroit de Barrow (22 ± 11 mg/kg). Les niveaux de mercure étaient en forte corrélation avec ceux du sélénium, avec un rapport molaire Hg/Se de 1,27.

TABLE 1

| Control Set → | $AFRD_i$ | $Larvae_i$ | $Ratio_i$ | $Pseudo\text{-}values_i$ |
|---|---|---|---|---|
| 1 | 39 | 8 | .205 | .073 |
| | 39 | 37 | .949 | 1.127 |
| | 15 | 12 | .800 | .668 |
| | 37 | 29 | .784 | .872 |
| | 24 | 33 | 1.375 | 1.246 |
| | 39 | 16 | .410 | .362 |
| 2 | 20 | 8 | .400 | .430 |
| | 20 | 13 | .650 | .617 |
| | 20 | 4 | .200 | .294 |
| | 30 | 5 | .167 | .141 |
| | 20 | 7 | .350 | .396 |
| | 30 | 3 | .100 | .073 |
| 3 | 30 | 10 | .333 | .328 |
| | 45 | 18 | .400 | .328 |
| | 24 | 29 | 1.208 | 1.093 |
| | 30 | 14 | .467 | .464 |
| | 30 | 9 | .300 | .277 |
| | 15 | 9 | .600 | .566 |

Another parameter of interest in this study is the variance of the estimators. The variance is necessary for determining the sensitivity of the statistical tests employed to detect a change. To study the distribution of the variance of each estimator, or the variability of the variance we applied the bootstrap technique to the ratio values and to the pseudo-values separately. Figure 1 is the distribution of 500 bootstrap samples of the variance of the average ratio estimator (top) and variance of the jackknife estimator (bottom).

Assuming the ratio values and the pseudo-values in Table 1 are from a Normal distribution, one can construct confidence interval estimates of the variance of each estimator. From Fig. 1, one can also find empirical confidence interval estimates of the variance. Table 2 compares the empirical intervals taken from Fig. 1 with calculated intervals assuming Normally distributed data.

It appears that the assumption of Normally distributed data is not appropriate, and that one should use the non-parametric bootstrap confidence intervals. Using the empirical confidence interval for the Jackknife variance one can determine the sensitivity of a statistical test to detect differences. Recognizing that the variance of our response of interest ranges from .059 to .180, allows us to calculate a range to the difference that can be detected between two exposure condition means. Following the procedure outlined in Snedecor and Cochran (1980) we can detect a difference between two exposure means of .26 to .46 for the ratio of offspring per female per day.

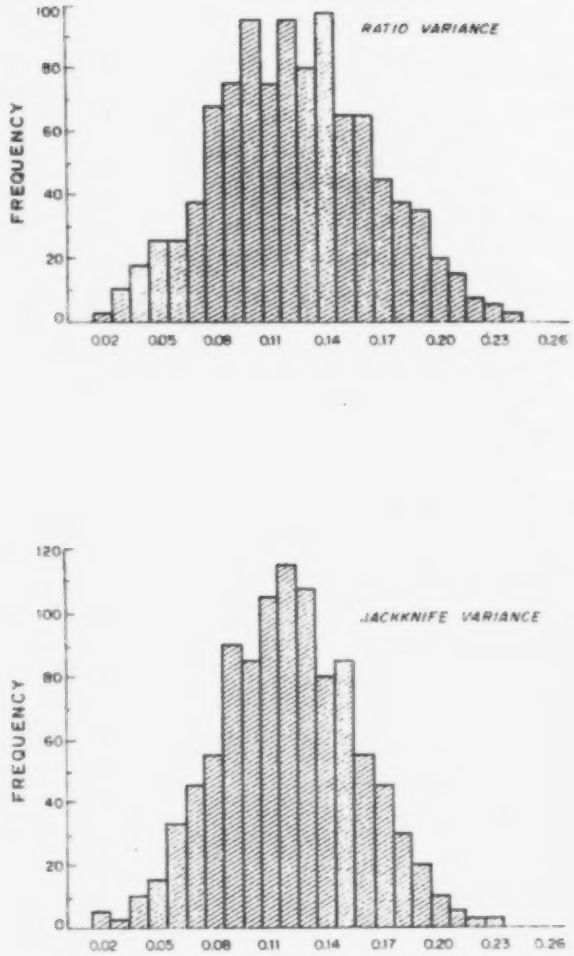

FIGURE 1 BOOTSTRAP DISTRIBUTION OF THE VARIANCE OF THE RATIO ESTIMATOR (Top) AND VARIANCE OF THE JACKKNIFE ESTIMATOR (Bottom)

TABLE 2

| Estimator | Empirical 90% C.I. | Normal 90% C.I. |
|---|---|---|
| Ratio Variance | .052 to .195 | .080 to .255 |
| Jackknife Variance | .059 to .180 | .078 to .249 |

Table 3 contains the data which we will use to illustrate the use of the rank transformation. These data came from a life-cycle bioassay in which M. bahia were exposed to sublethal concentrations of lead. The Rank variable is the rank of each ratio as ordered from smallest to largest. Ties are given their average rank.

Applying the parametric analysis of variance and Dunnett's procedure to the ratio, there was found to be no significant difference in the means of the control, 15 ppb, and 70 ppb exposure conditions. The 30 ppb, and 150 ppb exposure means were significantly less than the control mean ($p<.05$).

This conflicting conclusion is due mostly to one extremely large ratio in the 70 ppb exposure. Applying the rank transformation to the data and then going an analysis of variance and using Dunnett's (1955) procedure to compare each exposure treatment with the control treatment we get the results of Table 4. This grouping of the means is intuitively more appealing and is less affected by the one extreme value in the 70 ppb exposure.

While these methods, particularly the Bootstrap and Jackknife require considerable computations, they allow one to study the statistical properties of summary statistics other than the average. Knowing the distribution of the variance of an estimator as given in Figure 1, permits a researcher to determine how sensitive a testing procedure is at detecting some difference between experimental conditions. The Jackknife method generates a non-parametric standard error for any statistic, and the Bootstrap allows one to investigate the distribution of this statistic and generate empirical confidence intervals. Applying the rank transform to data allows the researcher to do non-parametric statistical tests using standard parametric statistical computer packages.

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TABLE 3

| Treatment | AFRD | LARVAE | RATIO | RANK |
|---|---|---|---|---|
| Control | 20 | 8 | .40 | 28 |
| | 20 | 13 | .65 | 29 |
| | 20 | 4 | .20 | 24 |
| | 30 | 5 | .17 | 22.5 |
| | 20 | 7 | .35 | 27 |
| | 30 | 3 | .10 | 19 |
| 15 ppb | 30 | 7 | .23 | 25 |
| | 30 | 1 | .03 | 16 |
| | 40 | 3 | .08 | 18 |
| | 20 | 6 | .30 | 26 |
| | 30 | 5 | .17 | 22.5 |
| | 20 | 3 | .15 | 20.5 |
| 30 ppb | 20 | 0 | 0 | 7.5 |
| | 30 | 0 | 0 | 7.5 |
| | 20 | 0 | 0 | 7.5 |
| | 10 | 0 | 0 | 7.5 |
| | 30 | 0 | 0 | 7.5 |
| | 20 | 3 | .15 | 20.5 |
| 70 ppb | 10 | 0 | 0 | 7.5 |
| | 10 | 0 | 0 | 7.5 |
| | 10 | 0 | 0 | 7.5 |
| | 20 | 14 | .70 | 30 |
| | 20 | 0 | 0 | 7.5 |
| | 20 | 0 | 0 | 7.5 |
| 150 ppb | 30 | 0 | 0 | 7.5 |
| | 20 | 1 | .05 | 17 |
| | 40 | 1 | .03 | 15 |
| | 40 | 0 | 0 | 7.5 |
| | 20 | 0 | 0 | 7.5 |
| | 20 | 0 | 0 | 7.5 |

TABLE 4

| Exposure | Average Rank |  |
|---|---|---|
| Control | 24.9 | * |
| 15 ppb | 21.3 |  |
| 70 ppg | 11.3 |  |
| 150 ppb | 10.3 |  |
| 30 ppb | 9.7 |  |

*Solid lines indicate means that are not significantly different from one another.

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# "MUSSELING" IN ON HEAVY METAL POLLUTION IN ESTUARINE ENVIRONMENTS USING PRINCIPAL COMPONENTS

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POPHAM, J.D. 1985. "Musseling" in on heavy metal pollution in estuarine environments using principal components. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 81-90.

Mussels Mytilus edulis, are used for monitoring estuarine pollution. The question remains as to how data obtained from the chemical analysis of mussels should be interpreted with respect to water quality. Normally a statistical procedure, usually regression analysis, has been involved and the results are used to derive conclusions on water quality. There is now abundant data demonstrating an interdependence among the variates, trace metal concentrations and especially body mass. Is an application of regression procedures the optimum way of treating the data, assuming that the only significant relationship occurring for metals is their dependency on mussel size. This paper discusses this problem in some detail.

POPHAM, J.D. 1985. "Musseling" in on heavy metal pollution in estuarine environments using principal components. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 81-90.

Les moules, Mytilus edulis, ont été utilisées pour surveiller la pollution des estuaires. La question reste de savoir comment interpréter les données obtenues par l'analyse chimique des moules, en ce qui concerne la qualité de l'eau. Normalement, une méthode statistique (analyse de régression) est utilisée, et les résultats ont servi à tirer des conclusions sur la qualité de l'eau. De nombreuses données montrent une interdépendance des variantes, des concentrations de métaux à l'état de trace et particulièrement de la masse corporelle. Si l'on estime que la seule relation significative applicable aux métaux est leur dépendance par rapport à la taille des moules, l'application des méthodes de régression est-elle le meilleur moyen de traiter les données? Tel est le problème analysé de façon détaillée par notre publication.

## DISCUSSION PAPER

The points expressed in this paper have been published previously or are now in press. An informal verbal presentation and discussion of them may help to indicate their strengths and weaknesses.

Mussels, Mytilus edulis, are used for monitoring estuarine pollution; hence the pun in the title. Nevertheless, the question still remains as to just how data obtained from the chemical analysis of mussels should be interpreted with respect to drawing inferences about water quality. Normally a statistical procedure, usually regression analysis, has been involved and the results used to derive conclusions on water quality since there is now abundant data demonstrating an interdependence among the variates, trace metal concentrations and especially body mass. Now, assuming that the only significant relationship occurring for metals is their dependency on mussel size, is an application of regression procedures the optimum way of treating the data?

The paradigm of an experiment for determining water quality would be to collect animals from a reference area and from one or more experimental areas of interest, plot the lines of best fit relating metal body burdens to size and finally compare the regressions using covariate analyses. The objective of using a covariate design is to remove any effect caused by differences among body weights. But such comparisons work only if the lines of regressions of all the samples have similar slopes since interpretation becomes very difficult if the relationship between the body burden of a metal and body mass varies for different populations (either in space or time). The problem is that slopes do not remain similar or constant and so covariate regression analyses becomes inappropriate.

Another issue which can be raised at this time but which is slightly off the main stream of thought is the use of the term "control" as in running a "control experiment" which can be defined as "an experiment in which the variable factors are controlled so as to make it possible to observe the results of varying one factor at a time". Do we really know what all the variables are much less how to vary them only one at a time? For this reason it may be wise to adopt the term "reference sample" in order to continually remind us that we are only scratching the surface in our understanding of "what is going on".

Let us get back to regression statistics per se. An alternate way of thinking about the relationships between sets of variables is to consider one set as representing a "predictor" variable and the other a "criterion" variable. It immediately becomes obvious that it is possible to have more than one predictor variable and hence use a procedure known as multiple regression analysis.

$$C = b_1P_1 + b_2P_2 + ... + b_nP_n + a$$

The objective of such regression equations is to "predict" the outcome given a particular set of data. An example of such an application follows. Mussels accumulate trace metals over time in proportion to the concentration of the metals in sea water and thus estimates of their body burdens should provide an indication of water quality. We attempted to determine if it were possible to produce equations to reflect this phenomenon using field-collected data. One of these equations is below:

$$\log (Cu_{SPM}) = -0.4523 - 0.2171 \log (Cu) - 0.3076 \text{ Day}' + 1.5212 \log (Fe) + 0.4566 \log \text{Dry Wt.} - 0.5682 \log (Sr) -0.1080 \log (Pb + 1)$$

where ($Cu_{SPM}$) is the concentration of copper as suspended particulate matter, where (Cu), (Fe), (Sr) and (Pb) are respectively the concentrations of Cu, Fe, Sr and Pb in the mussel, and where Dry Wt. and Day' are respectively the dry weight of the mussels and a factor reflecting the time of year of collection.

This model accounted for approximately 39% of the variance of log $Cu_{SPM}$ and thus is not very precise. The reason for this could be explained by the fact that the measured body burdens of metals in mussels are time-integrated values, while the measured concentrations of $Cu_{SPM}$ were instantaneous values. As a result low values for $Cu_{SPM}$ are overestimated and *vice versa*. Some factor reflecting "instantaneous" fluctuations is needed. For a number of reasons the only one we could come up with at the time was the concentration of $Fe_{SPM}$ in sea water. For example the original model predicting the concentration of $Pb_{SPM}$ without a variable reflecting instantaneous variation is:

$$\log (Pb_{SPM} + 1) = (-1.0487 + 0.1116 \log (Pb + 1) + 0.7097 (Fe) ).$$

This term in the brackets is a reflection of the "average" estimate of $Pb_{SPM}$ in sea water and accounts for only 22% of the variance of the criterion variable. By using a variate to reflect "instantaneous" readings differing from the average the following model was developed.

$$\log Pb_{SPM} + 1 = -1.6996 + 0.7195 (-1.0487 + 0.1116 \log (Pb + 1) + 0.7097 (Fe)) + 0.6523 (Fe_{SPM}),$$

and with it almost 37% of the variance of ($Pb_{SPM}$) could be accounted for. Results of a plot using this model shows that it tends not to so overestimate low values and under estimate high values compared with the first.

Now others have used multiple regression analysis to try and reveal which variates are the important ones as predictors by comparing the magnitudes of the regression coefficients. Under no circumstances should this ever be done. The beta weights in any regression equation take on their values to minimize the error sum of squares and thus maximize the predictive value of the whole model. I would like to give an example using artificial data to emphasize this very important point.

$$S_{pp} \cdot b = S_{pc}$$

$$\begin{array}{cc} 5325.084 & 5330.284 \\ & 5345.484 \end{array} \cdot \begin{array}{c} b_1 \\ b_2 \end{array} = \begin{array}{c} 5471.14 \\ 5469.14 \end{array}$$

$$b = S_{pp}^{-1} \cdot S_{pc}$$

$$\begin{array}{c} 5471.14 \\ 5469.14 \end{array}$$

$$b = \begin{array}{cc} 0.10043 & -\ 0.10015 \\ & 0.10005 \end{array} \cdot \begin{array}{c} 1.76278 \\ -0.73464 \end{array}$$

$$Y = -5.66 + 1.76 X_1 - 0.735 X_2$$

TABLE 1 ARTIFICIAL DATA USED TO CALCULATE A MULTIPLE REGRESSION EQUATION

| | Y | $X_1$ | $X_2$ |
|---|---|---|---|
| | 29 | 38.3 | 39.3 |
| | 15 | 16.1 | 15.1 |
| | 3 | 11.7 | 12.7 |
| | 79 | 82.9 | 81.9 |
| | 30 | 30.3 | 31.3 |
| | 29 | 29.3 | 28.3 |
| | 78 | 79.9 | 80.9 |
| | 15 | 23.4 | 22.4 |
| | 36 | 42.1 | 43.1 |
| | 40 | 45.4 | 44.4 |
| Sums | 354 | 399.4 | 399.4 |
| Means | 35.4 | 39.94 | 39.94 |
| SS | 5730.4 | 5325.084 | 5345.484 |

It is obvious that both $X_1$ and $X_2$ are excellent predictor variates of the criterion variate, Y; and yet $b_2$ is almost one-half the magnitude of $b_1$ and of opposite sign. Variate $X_2$ certainly is not "one-half" as important as variate $X_1$. The values of the regression coefficients are what they are because they minimize the error sum of squares for this set of data and for no other reason.

Finally, I would like to add a word about "Discriminant Analysis", i.e., the statistical technique usually used to optimally describe measured differences for two or more groups. This is the technique used, for example, to assign a human skull of unknown identity to a particular race with a known probability. This paradigm, however, is not the same as ours in which we want to determine whether or not data on a particular sample of mussels can be used to indicate if the mussels come from a body of water of good quality. Since it is practically impossible to collect all possible samples reflecting all possible types of poor water quality it is impossible to obtain the optimum discriminant functions. An illustrated example will be shown during the discussion.

All of the preceding leads up to the reason why we should seriously consider using Principal Component Analysis for "finding out what is going on with the data".

Principal components are a linear combinations of the original variables e.g. where

$$PC = b_1V_1 + b_2V_2 + \dots + b_nV_n$$

PC is the principal component, $b_1$ to $b_n$ are the principal component coefficients, and $V_1$ to $V_n$ are the standardized variates. The first principal component accounts for the highest proportion of the variance of the data set and each succeeding component accounting for successively smaller proportions of the total variance. Further, each principal component is uncorrelated with every other principal component. Generally only a few principal components are necessary to account for most of the variance of a data set, resulting in a parsimonious explanation of the data. Secondly, since the principal components are uncorrelated with one another they can be drawn at right angles to one another and the location or coordinates of any one specimen (its principal component scores) with respect to the origin is a simple Euclidean distance.

The magnitude and signs of the correlations between the PCs and the original variates reveals information about the interrelationship among the variates while calculations of principal component scores can be used to assess the quality of the environment.

Let me illustrate with an example.

For slightly over one year, mussels were obtained at monthly intervals from Rocky Point (49°17' N, 122°51'W), a location in Burrard Inlet, British Columbia previously shown to be relatively unpolluted compared to other areas in Burrard Inlet. Specimens were also obtained occasionally from other locations in British Columbia waters and all specimens analysed for trace element (Mn, Fe, Cu, Zn, Pb) concentrations by x-ray energy spectroscopy (XES).

The data were log transformed and then analysed using the statistical package BMDP 4M (factor and principal component analyses).

Although the effect of season could be taken into account in several ways, e.g. water temperature or the day of the year mussels were collected, we used a mathematical function to simulate seasonal effects, namely

$$\text{Day}' = \sin(\text{day}-364) + \cos(\text{day}-364)$$

where day is the day of the year of the collection (January 1, 1979 = day 1).

The matrix of correlations among the variates measured in this study for the 103 mussel samples collected from Rocky Point are listed in Table 2. Three principal components with eigenvalues greater than one were extracted from this matrix and account for slightly less than 71% of the total variance. The correlations between these principal components and the original variates after varimax rotation are listed in Table 3. Table 4 lists the means and standard deviations of the variates so that the normalized scores can be derived and Table 5 lists the value of the component score coefficients so that the component scores of specimens of mussels collected from other areas can be calculated.

Interpretation of what the principal components (PC's) represent is based on the values of the correlations between the PC's and the original variates (Table 3). For example, the variates expressing size of the mussel (i.e., dry weight and shell length) have high correlations with only the second PC. Since this PC is not highly correlated with any other variable it can be interpreted as representing mussel size.

The first PC can be interpreted, at least in part, as a variable accounting for seasonal changes in mussels since it is highly correlated with Day'. Since concentrations of Mn, Fe, Cu and Zn in mussels also are highly correlated with the first PC and only modestly correlated or poorly correlated with the other two it can also be interpreted as a variate accounting for much of the change in Mn, Fe, Cu and Zn concentrations in the mussels. This combination of the high correlation of variate, Day', and the modest to high correlations of the trace metals, excluding Pb, with the first PC indicates that the PC is

TABLE 2 CORRELATION MATRIX OF THE VARIATES USED IN DETERMINING WATER QUALITY

| | Dry Wt. | Day' | Mn | Fe | Cu | Zn | Pb | Water | Length |
|---|---|---|---|---|---|---|---|---|---|
| Dry Weight | 1.000 | | | | | | | | |
| Day' | -0.189 | 1.000 | | | | | | | |
| Mn | -0.356 | 0.535 | 1.000 | | | | | | |
| Fe | -0.428 | 0.674 | 0.673 | 1.000 | | | | | |
| Cu | -0.298 | 0.393 | 0.498 | 0.609 | 1.000 | | | | |
| Zn | 0.077 | 0.329 | 0.243 | 0.404 | 0.195 | 1.000 | | | |
| Pb | -0.010 | 0.132 | 0.138 | 0.214 | 0.224 | 0.262 | 1.000 | | |
| Water | -0.258 | 0.257 | 0.044 | 0.433 | 0.251 | 0.301 | 0.189 | 1.000 | |
| Length | 0.909 | -0.123 | -0.378 | -0.308 | -0.248 | 0.180 | 0.051 | -0.011 | 1.000 |

The definition of the variates in this table are as follows:

(1) dry wt. = dry weight of the meats
(2) Day' = function reflecting season of collection in mussels
(3) Mn to Pb = log transformed concentrations of the elements in mussels (mass of metal - unit dry mass of $\text{mussel}^{-1}$
(4) water = relative proportion of water in the mussels = $(1 - \frac{\text{dry wt.}}{\text{wet wt.}})$
(5) length = length of the right valve of the mussels

TABLE 3 CORRELATIONS BETWEEN THE ORIGINAL VARIATES (symbols as in Table 2) AND THE FIRST THREE PRINCIPAL COMPONENTS EXTRACTED FROM THE CORRELATION MATRIX (after varimax rotation), AND THE COMMUNALITIES OF THE VARIATES

| | Principal Components | | | Communalities[a] |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| Dry Wt. | -0.206 | 0.942* | -0.119 | 0.944 |
| Day' | 0.795* | 0.015 | 0.160 | 0.658 |
| Mn | 0.857* | -0.216 | -0.087 | 0.789 |
| Fe | 0.808* | -0.246 | 0.362 | 0.844 |
| Cu | 0.647* | -0.216 | 0.229 | 0.518 |
| Zn | 0.426* | 0.338 | 0.524* | 0.570 |
| Pb | 0.134 | 0.087 | 0.580* | 0.362 |
| Water | 0.058 | -0.198 | 0.848* | 0.762 |
| Length | -0.200 | 0.929* | 0.123 | 0.918 |

* values used in interpretation of the PC's

[a] communalities represents the proportion of variate accounted for by the three principal components.

accounting for the seasonal changes in Mn, Fe, Cu and Zn in mussels. Independent of this seasonal effect component is another represented by the third PC, which shows moderately high correlations between it and the concentrations of Zn and Pb as well as with the concentration of body water in the mussel. Since the correlations all have the same sign the component can be interpreted as indicating that those mussels with high body burdens of lead and zinc also tend to have high concentrations of body water. That is to say, these mussels have a high wet weight to dry weight ratio.

The results show that two principal components, the first and the third, account for most of the variance in the measured trace metal concentrations in the mussels collected from Rocky Point. The question now remains as to how these two principal components can be of value for determining trace metal pollution in estuarine waters. For such a determination we calculate the scores for the first and third principal components for the mussels using the regression coefficients (Table 5).

These scores act as the coordinates of the specimen in the plane denoted by the first and third PCs. If the specimen is close to the intersection of the two PCs then it resembles the specimens used to derive the PCs. Since the values are standardized, probabilities of similarity can be assigned to this distance as each unit represents a standard deviation unit. Specimens with a distance greater than two standard deviation units have a less than 5% probability of resembling the reference sample.

TABLE 4 STATISTICS (mean (x) and Standard Deviation (s)) OF TRANSFORMED VARIATES NECESSARY FOR DERIVING STANDARDIZED SCORES (Z)

| Variates[a] | Mean | Standard Deviation |
|---|---|---|
| Dry Weight | -0.80694 | 0.42921 |
| Shell Length | 1.51787 | 0.13546 |
| Day' | 0.06656 | 1.07038 |
| Metal Concentrations | | |
| Mn[b] | 0.65490 | 0.55395 |
| Fe | 2.53977 | 0.22879 |
| Cu | 0.93294 | 0.12401 |
| Zn | 2.71174 | 0.18713 |
| Pb[b] | 0.39742 | 0.28676 |
| Water Content[c] | -0.08440 | 0.01406 |

[a] variate = log variate

[b] Pb = log (Pb + 1), Mn = log (Mn + 1)

[c] Water Content = $\log\left(1 - \frac{\text{Dry Weight}}{\text{Wet Weight}}\right)$

TABLE 5 PRINCIPAL COMPONENT COEFFICIENTS FOR DERIVING SCORES FOR THE FIRST AND THIRD PRINCIPAL COMPONENTS OF THE MUSSELS COLLECTED FROM ROCKY POINT

| Variates | Coefficients | |
|---|---|---|
| | PC 1 | PC 3 |
| Dry Weight | 0.09091 | -0.10741 |
| Shell Length | 0.02907 | 0.07856 |
| Day' | 0.35427 | -0.08337 |
| Concentration of Metals | | |
| Mn | 0.40920 | -0.27323 |
| Fe | 0.26338 | 0.08826 |
| Cu | 0.22371 | 0.02461 |
| Zn | 0.13319 | 0.26893 |
| Pb | -0.06856 | 0.40561 |
| Water Content | -0.22452 | 0.65259 |

## A WORKED EXAMPLE

1. Collect specimens.
2. Measure values for each of the variates and calculate PCs as in 3 below.
3. Let us use a specimen collected from a wharf in an industrial area in Burrard Inlet.

| Variate | Raw Data | Transformed Data | Standardized[a] Score | PC1[b] ×Coefficients | PC1 |
|---|---|---|---|---|---|
| Dry Weight | 0.094 | -1.027 | -0.512 | 0.091 | -0.047 |
| Day of Collection | 93 | 1.017 | 0.888 | 0.354 | 0.314 |
| Metal Concentrations | | | | | |
| Mn | 32 + 1 | 1.519 | 1.559 | 0.409 | 0.638 |
| Fe | 633 | 2.801 | 1.144 | 0.263 | 0.301 |
| Cu | 49 | 1.690 | 6.106 | 0.224 | 1.368 |
| Zn | 415 | 2.618 | -0.501 | 0.130 | -0.067 |
| Pb | 12 + 1 | 1.114 | 2.499 | -0.069 | -0.172 |
| Water Content | 0.855 | -0.068 | 1.164 | -0.225 | -0.262 |
| Shell Length | 27 | 1.431 | -0.645 | 0.029 | -0.019 |
| Score for PC1 = | | | | | 2.054 |

[a] e.g. for Day of Collection = $\frac{1.017 - 0.067}{1.070}$ where

0.067 and 1.070 are the mean and standard deviation of the variate from the reference area.

[b] see Table 5

4. Similarly the value for PC3 can be determined. (PC3 = 1.39)
5. Calculate distance of specimen from origin:

$$D^2 = 2.054^2 + 1.39^2$$

$$= 2.48$$

6. Since D = 2.48 is between 2 and 3 standard deviation units from the origin we can conclude that the probability that the specimen is question resembles the specimens of the reference collection is between 5 and 1%.

7. The exact probability value can be determined using the chi-square value e.g.

   $X^2 = D^2 = 6.151$

8. The probability of $X^2 = 6.151$ with 1 d.f. is 1.313%.

# MODELLING THE LONG TERM FATE AND EFFECTS OF ANTHROPOGENIC POLLUTANTS ON THE NORTH AMERICAN CONTINENTAL SHELF

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REED, M., T. ISAJI, J. ROSEN, and S. HURLBUT. 1985. Modelling the long term fate and effects of anthropogenic pollutants on the North American Continental Shelf. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 91-124.

This paper describes a current project designed to assess the ecological effects of various hypothetical ocean disposal policies. The model system estimates pollutant trajectories, areas of influence, and biological impacts including bioaccumulation. The project involves the formulation of a quasi-steady state (seasonal) two-layer hydrodynamic transport model covering the United States East, Gulf of Mexico, and West coast continental shelves, excluding Alaska, and a set of simple ecosystem box models for impact estimation. Model ecosystems are associated with specific sets of transport model grids (e.g. Georges Bank, New York Bight, Eastern Gulf of Mexico). Using approximate exposure-response relationships between contaminants and organisms or trophic levels, plus food web linkages, ecosystems effects are estimated in terms of biomass reductions and bioaccumulation. Harvesting rates will give a measure of human exposure levels.

An example application of the system to a deep water dumpsite is presented and discussed.

REED, M., T. ISAJI, J. ROSEN, and S. HURLBUT. 1985. Modelling the long term fate and effects of anthropogenic pollutants on the North American Continental Shelf. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 91-124.

Cette communication est consacrée à la description d'un projet actuel visant à évaluer les effets écologiques de diverses politiques hypothétiques sur les rejets en mer. Ce modèle permet d'évaluer les trajectoires des polluants, les zones d'influence et les effets biologiques, y compris la bio-accumulation. Le projet comprend la formulation d'un modèle de transport hydrodynamique à deux couches en état de quasi-équilibre (saisonnier), couvrant le plateau continental de l'Est des États-Unis, du golfe du Mexique et de la côte ouest, à l'exclusion de l'Alaska, et un jeu de modèle simples d'écosystèmes ayant pour but l'estimation des effets. Les écosystèmes modèles sont associés à des jeux spécifiques de réseaux de modèles de transport (par exemple banc de George, baie de New York, partie est du golfe du Mexique). Grâce à des rapports exposition-réaction approximatifs entre contaminants et organismes ou niveaux trophiques, plus les réseaux trophiques, les effets sur les écosystèmes sont estimés en termes de réduction de biomasse et de bio-accumulation. Les taux d'exploitation permettront de mesurer le niveau auquel l'homme est exposé.

A titre d'exemple, on présente et on analyse une application du système à un lieu de rejets en eau profonde.

# INTRODUCTION

The development of rational waste disposal policy decisions aimed at minimizing public health hazards, ecosystem degradation, and disposal costs necessitates the quantification of environmental risks, so that alternative disposal scenarios can be objectively compared. This paper describes the progress of an on-going project to formulate a marine pollutant fates and effects model system. The model is intended to supply quantified impact and hazard assessment estimates to support the development of long term waste disposal policies in the United States.

A model system of this type must satisfy several fundamental criteria. First, it must be affordable, both in its formulation and application stages. It should therefore tend towards simplified and generic rather than complex and detailed formulations. This parsimonious bent must be balanced, however, by the need to adequately represent the important processes governing system behavior such that model output is representative of the real system, and is useful to decision makers. Second then, model outputs must be meaningful measures of risk. Pollutant concentration histories and residence times in the water column and sediments, human exposure levels through food web biomagnification, and commercial fishery yield reduction levels are examples of useful impact measures which are estimable with the system being developed here. Third, it should be possible to evaluate uncertainties associated with model output. Although environmental uncertainty has not yet been incorporated here, an appropriate stochastic methodology has already been established (Reed et al. 1983b, Lorda et al. 1983). Fourth, the model system must be resolved in time and space scales commensurate with the problem to be addressed. Waste disposal policies can remain in force for 10 or 20 years. Given that mean transport rates on the North American continental shelf are on the order of 10 cm/sec (or about 3000 km/yr), spatial dimensions which are only shelf-wide implicity assume the open ocean to be an infinite sink. The long time scales imply that mean velocity fields at seasonal frequencies will be adequate for transport estimates, assuming that aperiodic events occurring at higher frequencies are properly parameterized.

The model system being developed here consists of a series of water column and sediment pollutant transport and fate and ecosystems submodels, which are coupled to simulate specific geographic waste disposal scenarios.

The water column pollutant transport models use seasonal or annual estimates of mean velocity fields and water column chemistry to estimate the hydrodynamic transport of pollutants. Ultimate disposition from this model is either to the sea floor or out the open boundaries, or via decay processes.

Two hydrodynamic models are being developed, including a three dimensional finite difference algorithm for solution of the momentum and mass conservation equations, and a data-based empirical approach similar to that used by Reed (1980) and Spaulding et al (1982) for oil spill fishery impact assessment. The time-space distribution of a pollutant in the water column and on the sea floor is computed by the transport models, using adsorbed-dissolved partitioning and estimates of particulate settling velocity distributions.

The sediment model describes pollutant incorporation into and release from the sediments as a function of time. The dynamic transfer of pollutants into the sediments is estimated with a one dimensional numerical solution to the equation for diagenesis (Duursma and Smies, 1982).

The basic ecosystems model is a spatially averaged set of coupled ordinary differential equations, combined with formulations of physiological and metabolic processes. The ecosystems model receives input from the water column and sediment models in the form of pollutant concentrations. These concentrations determine the pollutant concentrations at the lower trophic levels, and bioaccumulation is computed as a function of diet and octanol-water partitioning throughout the food web.

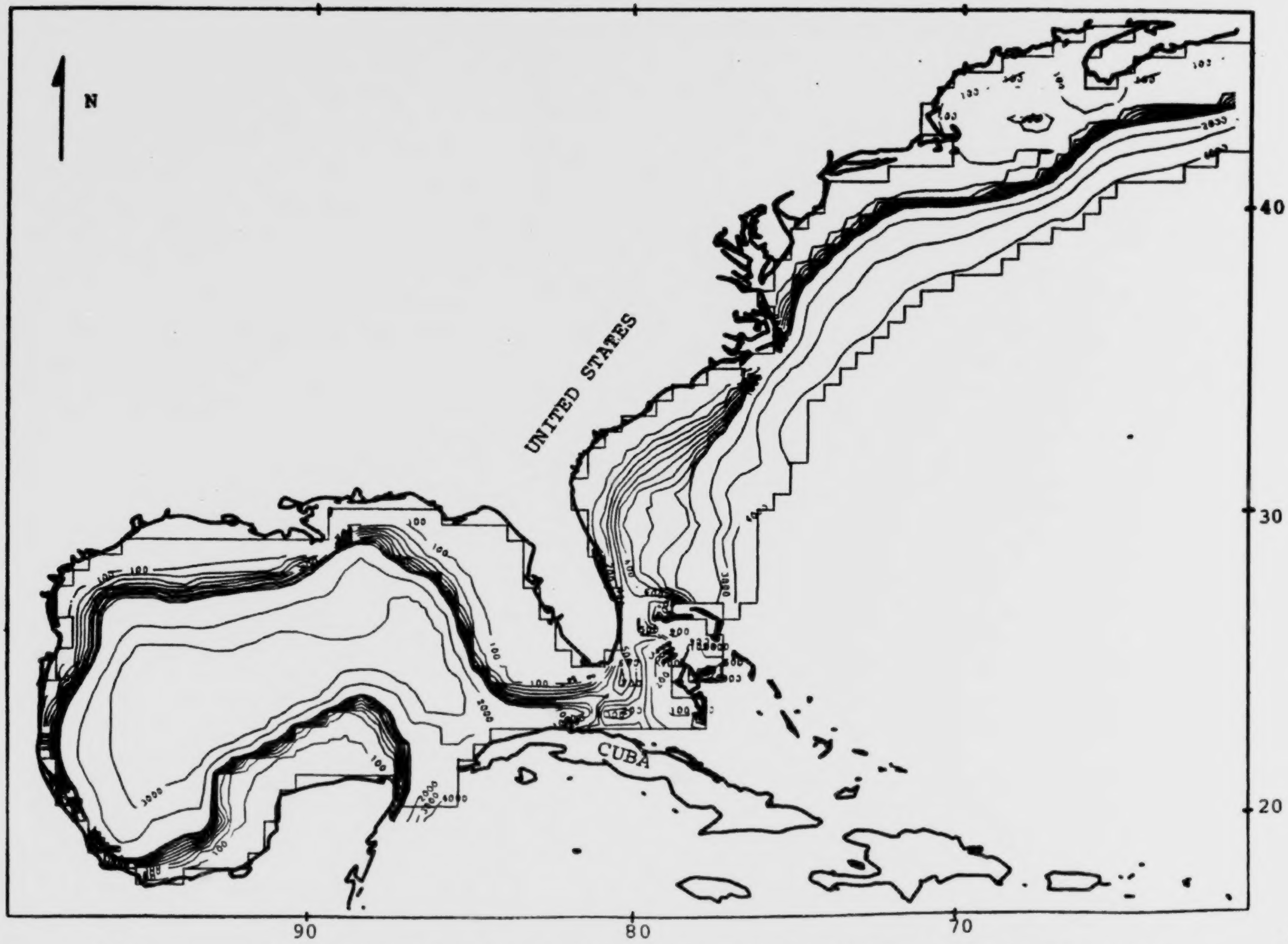
Each of these components will be described in more detail below.

## Hydrodynamic Models

The spatial scales of interest are on the order of $10^3$ km. It is therefore generally necessary to perform hydrodynamic computations on a spherical grid system to hold down computational error at latitudes far removed from the central numerical grid cells. Within this restriction, two separate hydrodynamic transport models are being applied to the long term pollutant transport problem. Each has its own advantages and drawbacks, and it is intended that the simplest approach which results in satisfactory estimates of long term pollutant transport will be selected and carried forward in future system development.

The more advanced approach applied here is a full three dimensional model, in which the mass, momentum, salt and heat conservation equations are solved by a forward in time, centered in space finite difference scheme. The vertical variations in horizontal velocity are described in terms of an expansion of Legendre polynomials, solved using the Galerkin weighted residual method. Details of this modeling approach can be found in Isaji et al. (1982). A preliminary test of the spherical coordinate code has been performed for the Gulf of Mexico and East Coast of the United States, using a resolution of 1/4 degree in the horizontal and the bathymetry show in Figure 1. Taking estimates from the literature, mass flux is specified at all open boundaries, such that mass is conserved globally, and the model is then run until the internal field has reached an equilibrium configuration. The resultant surface current field from this simple test is shown in Figure 2. Erroneous circulation in the Gulf of Mexico results primarily from elimination of wind forcing for this test case. Although this approach can ultimately supply the best description of the physics governing the transport of pollutants on the continental shelf, both computational and boundary data constraints restrict its repeated application for numerous scenarios. The three dimensional output, given better boundary conditions, will eventually supply a measure of adequacy for the simpler method of transport estimation described below.

The data based modeling approach uses empirical measures of mean seasonal currents from a variety of sources to provide the basis for transport estimates. Since mean surface and bottom currents may be quite different, it will be necessary to apply a two-layered representation. The basic data source for surface currents is the series of atlases produced by the Naval Oceanographic Office, based primarily on ship drift observations. Current meter and ocean drifter data summaries (e.g. Bumpus and Lauzier, 1965) have also been incorporated wherever possible. Summer and winter surface current data sets are shown in Figures 3 and 4.


N
UNITED STATES
CUBA
100
200
500
1000
2000
3000
4000
90
80
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40
30
20

FIGURE 1 BATHYMETRY, COASTLINE, AND MODEL GRID OUTLINE FOR THE GULF OF MEXICO AND EAST COAST

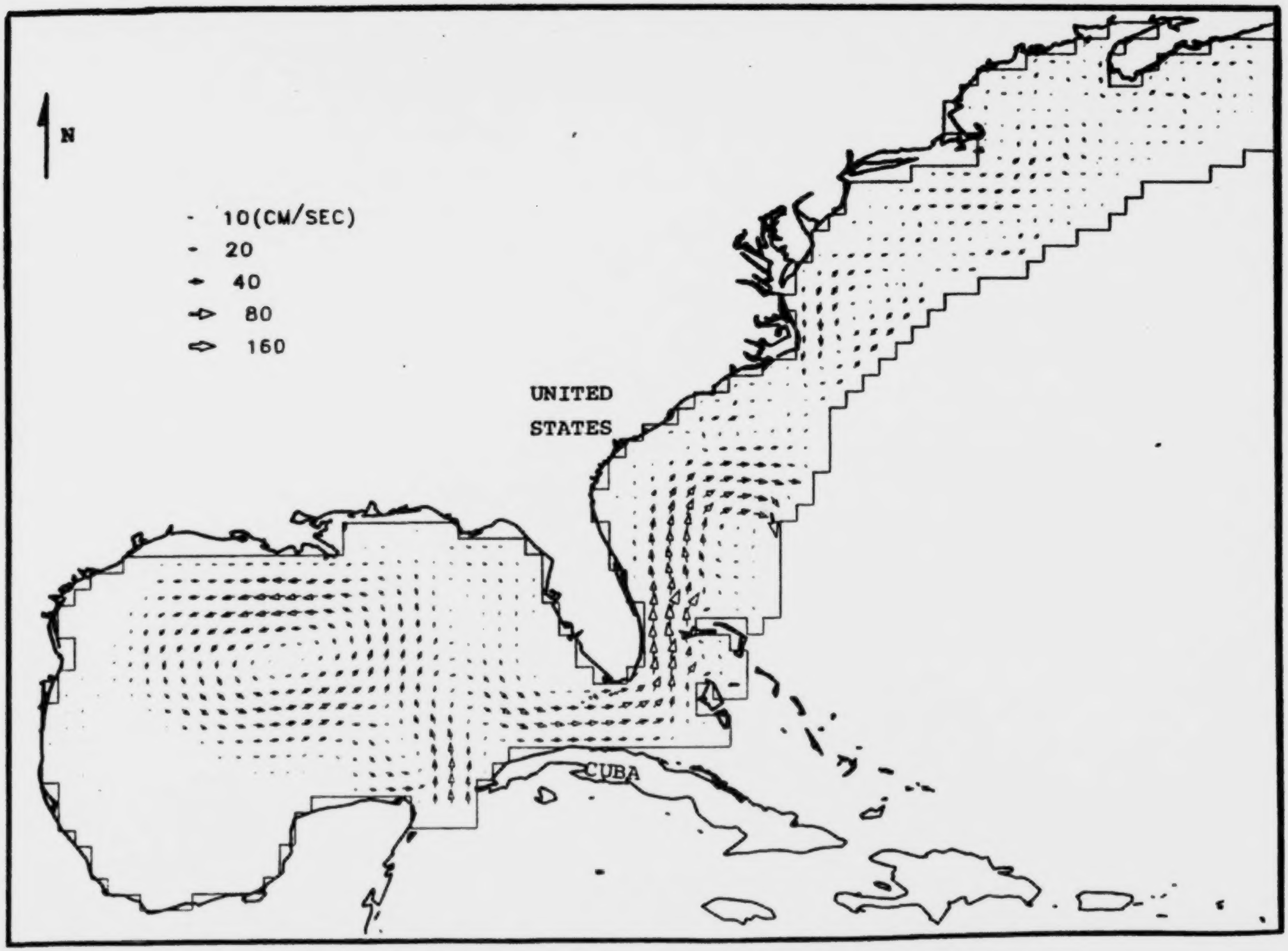

N
10(CM/SEC)
20
40
80
160
UNITED STATES
CUBA

FIGURE 2 PRELIMINARY OUTPUT FROM THREE DIMENSIONAL SPHERICAL COORDINATE HYDRODYNAMIC MODEL. ERRONEOUS CIRCULATION IN THE GULF OF MEXICO RESULTS FROM ELIMINATION OF WIND FORCING FOR TEST CASE. BOUNDARY CONDITION PROBLEMS ARE APPARENT AT THE GULF STREAM EXIT

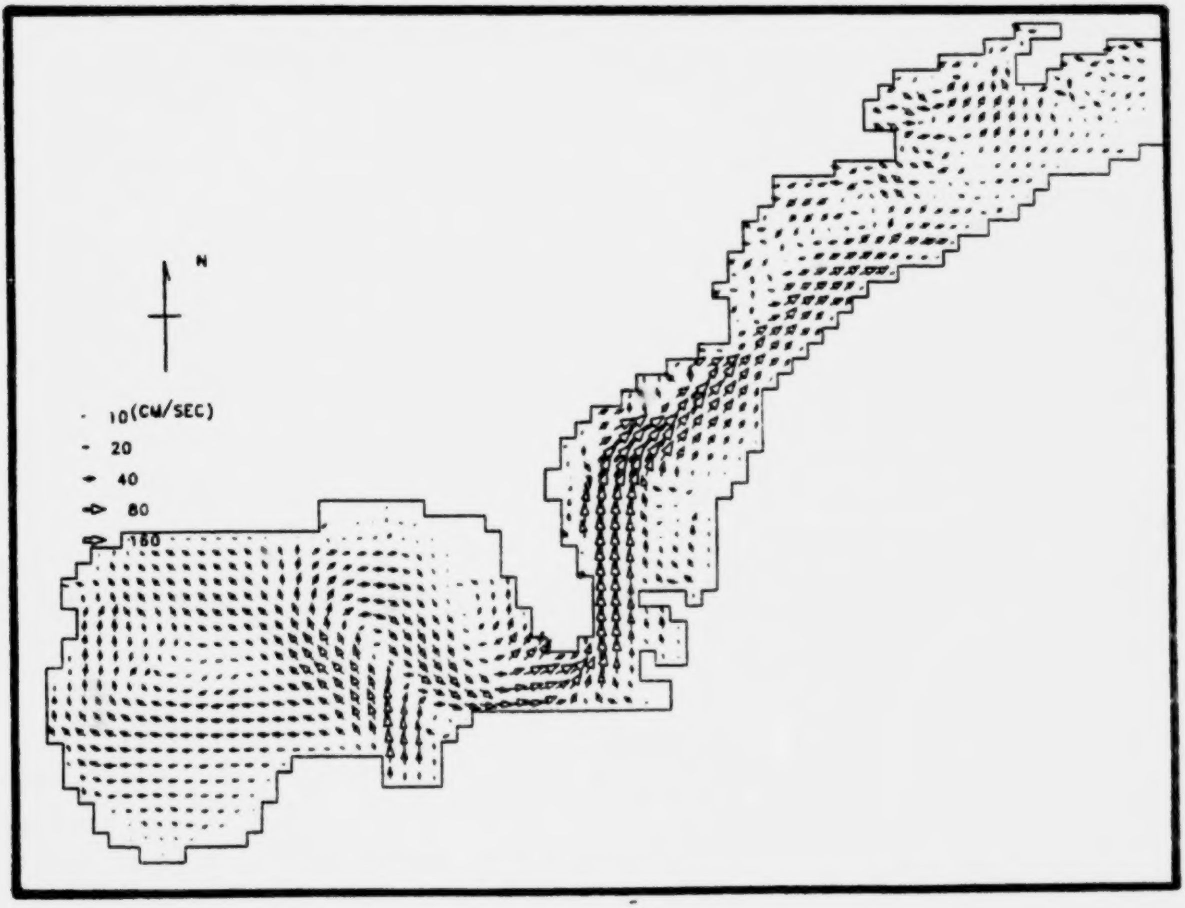

N
10(CM/SEC)
20
40
80
160

FIGURE 3 SUMMER SURFACE CURRENT DATA SET FOR GULF OF MEXICO AND EAST COAST

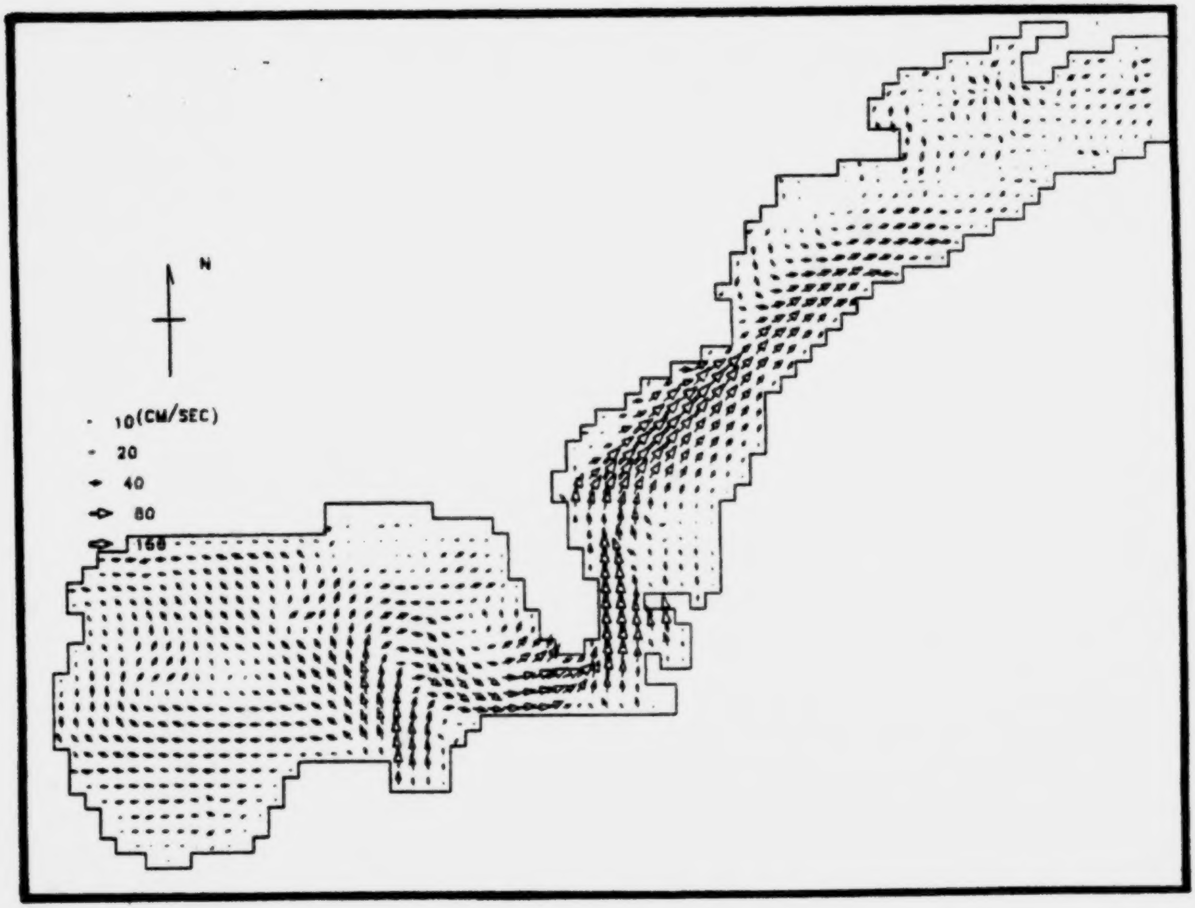

N
10(CM/SEC)
20
40
80
160

FIGURE 4 WINTER SURFACE CURRENT DATA SET FOR EAST COAST AND GULF OF MEXICO

Observations of long term mean bottom currents are much sparser in the literature, since current meter deployments are virtually the only source of information. The reduction and digitization of available information is presently in progress.

Seasonal near-surface and near-bottom current data sets will be produced in this way for the East, Gulf and West Coasts of the United States. These data sets will then be used to supply velocity information to the two layer pollutant transport model. Verification will be attempted by comparing modeled and observed seasonal salinity distributions. Comparisons will also be made with output from the three dimensional numerical model described above.

**Pollutant Dynamics: Water Column and Sediments**

A majority of the anthropogenic pollutants of concern in the marine environment, including hydrocarbons, heavy metals, radionuclides, chlorinated hydrocarbons and other organic compounds are readily adsorbed onto suspended particulate matter and eventually settle to the sea floor. It is therefore important to include in the model system adsorption - desorption and settling dynamics. This is necessary not only to properly simulate pollutant transport and fate, but also to estimate pollutant concentrations within biological trophic compartments. Food is generally a more important factor in bioconcentration calculations than water (Fowler 1982; O'Connor 1983), and it is therefore important to provide reasonable estimates of biologically available concentrations at the primary and secondary production levels, to achieve correct estimates of concentrations at the higher trophic levels.

Total pollutant concentrations are resolved into adsorbed and dissolved phases assuming equilibrium partitioning (e.g. Bierman and Swain, 1982):

$$f = 1/(K_p C_{ss} + 1)$$

in which $f$ = fraction of pollutant is dissolved form, $K_p$ is a dimensionless partition coefficient and $C_{ss}$ is the suspended solids concentration (mass of solids/mass of solution). Voice et al (1983) report evidence that partition coefficients are inversely dependent on the solids concentration, but because the general relationship remains uncertain we use a constant value of $K_p$ modified by the estimated weight fraction of solids comprised of organic carbon.

The particulate adsorption and settling processes in the model system result in a long term flux of pollutants from the water column to the sea floor. The distribution of settling velocities is taken from O'Connor et al (1983) for sewage sludge, and from Hawley (1982) and Carder et al (1982) for natural particulates and aggregates. We assume that sediment resuspension and transport will only occur at depths less than 100 meters, where wave and storm-induced effects become important. At greater depths, pollutants are removed from the water-sediment interface by (1) dissolution into the water column, (2) bioturbation, and (3) advection - diffusion into the sediments via the pore water. The governing differential equation for vertical pollutant transport within the sediments, inclusive of a first order decay term is:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial z^2} - w\frac{\partial C}{\partial z} - kC$$

in which C is pollutant concentration, D is a diffusion coefficient which includes bioturbation effects, t is time, z is distance positive downward into the sediments, w is interstitial water velocity, and k is the pollutant decay rate.

### Ecosystem Dynamics

Ideal ecosystems models for pollutant impact assessment include spatial dynamics of the major biological species of interest. Multi-species models with spatial resolution have proven computationally cumbersome and relatively data intensive (e.g. Reed and Balchen 1982, Laevastu et al 1979). Such complex models are also very difficult to apply to new geographic regions. We therefore make the simplifying assumption that, in the long term, the various trophic components of a given ecosystem can be modeled as homogeneously distributed in the horizontal dimension over a specified set of hydrodynamic grid cells. Associations with locations in the vertical (i.e. euphotic zone, lower water column, sediments) are implicitly maintained relative to predation, nutrient recycling, and pollutant exposure computations.

A second major simplification has been the generic representation of ecosystems by trophic compartments. These compartments, and the relationships among them, are shown in Figure 5. A series of adjacent but biologically distinct ecosystems may be modeled to evaluate the extent of impacts by a single ocean dumping policy. Migratory components (e.g. bluefish or grey whales) may be passed from the physical domain of one ecosystem to another as a deterministic function of time.

The biomass in the $i^{th}$ comaprtment, $B_i$, is governed by an ordinary differential equation of the form

$$\frac{dB_i}{dt} = B_i\,(-FMRT_i - PMRT_i - \sum_{j=1}^{s} P_{ji}\,B_j) + \text{GROWTH} + \text{RECRUITMENT}$$

in which $FMRT_i$ and $PMRT_i$ are the fishing and pollutant-induced instantaneous mortality rates. The $P_{ji}$ terms are measures of the predation rate of the $j^{th}$ compartment on the $i^{th}$, with dimensions of per unit biomass per unit time.

Phytoplankton growth is controlled by a deterministic sinusoidal function representing seasonality of abiotic factors (temperature and light). Thus the potential for phytoplankton growth is maximal during the summer and minimal during the winter. Actual phytoplankton production is then limited by the availability of nitrogen in the euphotic zone. This limitation is incorporated via a Michaelis-Menten formulation (e.g. Ebenhoh, 1980). Nitrogen is supplied by upwelling from deeper waters, and from biological excretion (Figure 5). Phytoplankton biomass growth is then further limited by zooplankton grazing.

Growth of faunal biomass is governed by a generic physiological model (Figure 6), whose parameters can be adjusted to account for differences in metabolic and generation times among the various ecosystem levels. The instantaneous rate of biomass flux into a compartment is calculated using the predation matrix elements $P_{ij}$:

$$\text{Predation} = \sum_{j=1}^{s} P_{ij}\,B_iB_j$$

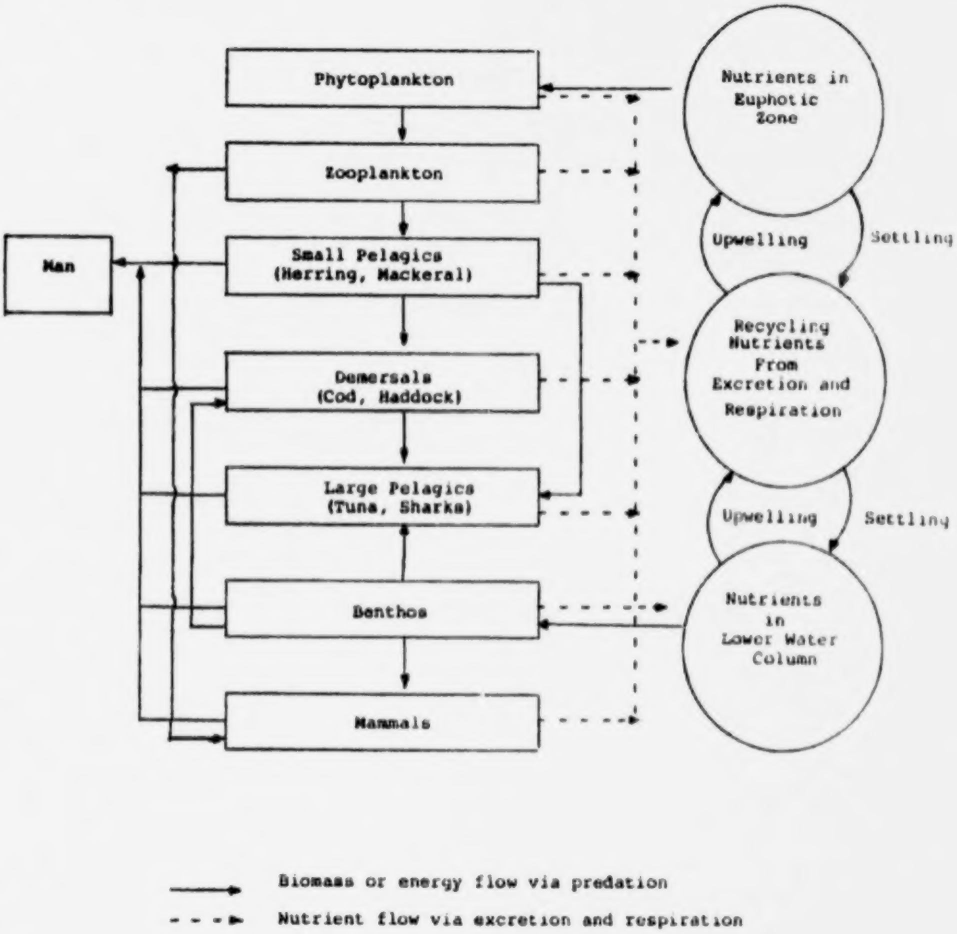

Phytoplankton
Zooplankton
Man
Small Pelagics (Herring, Mackeral)
Demersals (Cod, Haddock)
Large Pelagics (Tuna, Sharks)
Benthos
Mammals
Nutrients in Euphotic Zone
Upwelling
Settling
Recycling Nutrients From Excretion and Respiration
Upwelling
Settling
Nutrients in Lower Water Column
Biomass or energy flow via predation
Nutrient flow via excretion and respiration

FIGURE 5 GENERIC ECOSYSTEM MODEL STRUCTURE, SHOWING DIRECTION OF NUTRIENT FLOWS

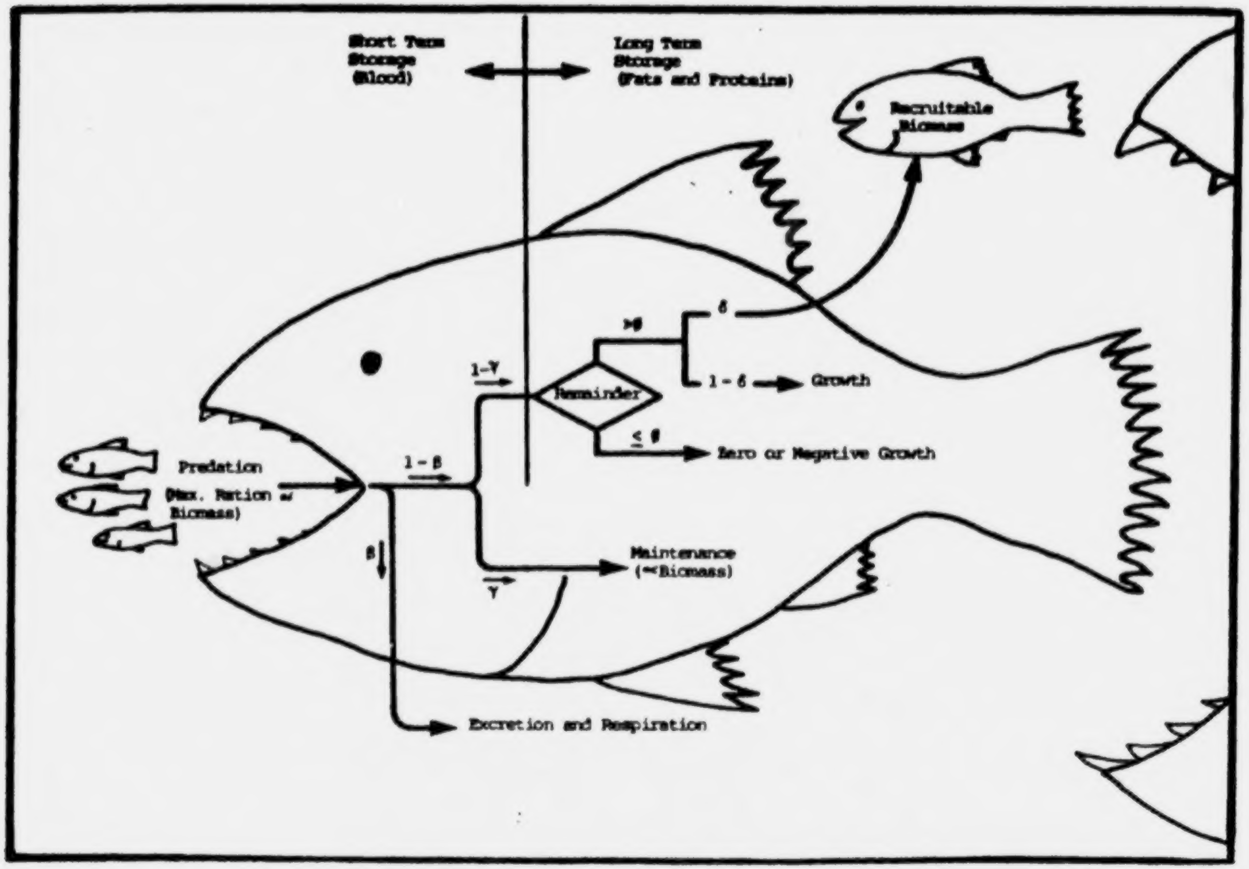

Short Term Storage (Blood)
Long Term Storage (Fats and Proteins)
Recruitable Biomass
Predation (Max. Ration ∝ Biomass)
$1-\beta$
$\beta$
$1-\gamma$
$\gamma$
Remainder
$>0$
$\delta$
$1-\delta$
Growth
$\leq 0$
Zero or Negative Growth
Maintenance (∝ Biomass)
Excretion and Respiration

FIGURE 6 PHYSIOLOGICAL SUBMODEL SCHEMATIC FOR FAUNAL TROPHIC COMPARTMENT REPRODUCTION, GROWTH, AND NUTRIENT RECYCLING

in which $P_{ij}$ is the predation rate of the $i^{th}$ trophic compartment on the $j^{th}$, s is the number of trophic levels, and $B_i$ and $B_j$ are the relative biomass levels in each compartment. The predation rates are subject to the limitation of a maximum instantaneous ratio, which is assumed linearly proportional to the biomass present in the predating compartment. In other words, codfish cannot eat more than, say, 1 kg of prey per 10 kg cod per day. If the specified satiation level is exceeded, predation on all affected compartments is reduced such that the ration limit is satisfied.

Predated biomass entering the metabolic submodel for a given trophic level (the $i^{th}$) is immediately reduced by a factor $\beta$ (Figure 6), representing excretion and respiration losses, and including estimates of swimming energy costs (Jones and Johnston, 1977). A second reduction factor $\gamma$ is then applied to account for maintenance needs, which are assumed linearly proportional to biomass present. Excretion, respiration and maintenance losses are added to the nutrient recycling compartment (Figure 5). Any predation input in excess of the above two needs is then divided between growth and reproduction. A fraction $\delta$ is added to recruitable biomass storage for the trophic compartment in question. This recruitable biomass is returned to the parent compartment at a rate which typifies that trophic level. The remaining fraction $(1-\delta)$ is added directly to $B_i$. If $\delta$ inputs are insufficient to meet maintenance needs, then negative growth (weight loss) occurs. The biomass loss is then added to the recycle compartment. Thus biomass is conserved within the overall system.

## Ecosystem Impacts

Two types of impact are estimable from the model system:

(1) bioaccumulation,
(2) biomass reductions through lethal or sublethal toxic effets on parent stock or recruitable biomass.

The two may be coupled, if bioaccumulation results in pollutant levels which are toxic in higher trophic compartments.

Bioaccumulation is computed in parallel with the physiological model of Figure 6, which shows metabolic processes associated with both short term and long term storages. Figure 7 gives a schematic for the bioaccumulation calculations within a given compartment. The pollutant concentration in short term storage for the $i^{th}$ compartment is assumed as a minimum to be in chemical equilibrium with the dissolved phase in the water (or sediments for benthos). If the pollutant concentration in the food exceeds that in the environment the short term storage is set equal to this new level. Long term storage is connected to short term storage as shown in Figure 7, by an assimilation rate $f_1$ and a depuration rate $f_2$ (from fats and proteins to blood). Elevated concentrations in the blood relative to the environment result in depuration to the environment at the rate $f_3$. The rates $f_1$, $f_2$, and $f_3$ are both pollutant and species (trophic compartment) specific.

Biomass reductions through pollutant induced mortality are simulated through the second term ($PMRT_i$) in Eq. 2. Bioassay results are used to derive mortality response curves for each pollutant. In general, the time and space scales of the problems addressed here inherently focus on long term chronic rather than short term acute toxic effects. As Vandermeulen and Capuzzo (1983) point out, our understanding of the full range of

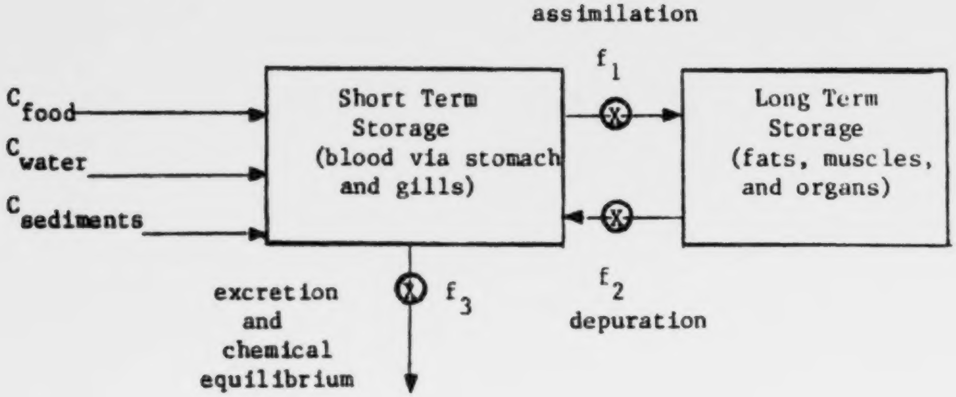

assimilation
$f_1$
$C_{food}$
$C_{water}$
$C_{sediments}$
Short Term Storage (blood via stomach and gills)
Long Term Storage (fats, muscles, and organs)
excretion and chemical equilibrium
$f_3$
$f_2$
depuration

FIGURE 7 METABOLIC SUBMODEL SCHEMATIC FOR BIOACCUMULATION ESTIMATION

sublethal disruptive responses at each trophic level is poor relative to our knowledge of acute toxic effects. It is therefore necessary in general to extrapolate low level effects from what little chronic biassay data may be available.

## Preliminary Example Application

The United States Environmental Protection Agency (EPA) is presently considering Deep Water Dumpsite (DWD) - 106, located at about the 2200 meter bathymetric contour southeast of New York City, as a receiving site for municipal sewage sludge, as well as aqueous industrial wastes for which it is now designated (Paul et al., 1983). The initial approval of the site for sewage sludge disposal will probably be for three years, to allow time to study the impacts of the action. A decision for the longer term policy could then be made on the basis of these findings.

This proposed disposal policy presents a realistic and timely scenario for a preliminary application of the impact assessment model system described here. This system has not yet been fully calibrated or verified, but the numerous earlier field and model studies which have been performed to assess DWD-106 Site impacts (e.g. O'Connor et al. 1983, Paul et al. 1983, Ketchum et al. 1981; Csandy et al, 1979) can be used to assist in the calibration process.

The velocity field used for this example application is the average of the summer and winter surface data sets shown in Figures 3 and 4. Use of a steady velocity field for a multi-year simulation allows for the solution to approach some quasi-equilibrium in the water column, as inputs become balanced by pollutants decay and removal to the sediments and out the open boundaries, and allows a simpler test of system conservation properties than a more realistic seasonally varying current field.

Although the model system can simulate multiple pollutants, we seek to verify system behavior subject to single contaminants before attempting synergistic effects. PCB has been selected as the sewage sludge constituent of interest for this example. It is assumed that only particulate-associated PCB will settle. The decay rate has been set to 0.0 for this test case. We use a short policy time horizon of 6 years, and carry the system through the loading and unloading cycle, subject to the input parameters given in Table 1.

Figures 8a-e show the time history of PCB concentrations averaged over the top 100 meters of the water column. The 100 meter depth is used here rather than the entire water column to be consistent with previous work (e.g. Csanady et al, 1979), and because this represents a year-round average depth for the pycnocline at this site (Orr and Baxter, 1983). For the physical and chemical system parameters specified in Table 1, the PCB concentrations in the water column reach an equilibrium level after about 1.5 years. At this time, losses out the open oceanic boundary to the east and to the sea floor through particulate scavenging and settling very nearly balance the input rate of 7.6 kg PCB/day. This 1.5 year time scale can therefore be taken as indicative of the water column residence time in the area (i.e. Cape Hatteras to Cape Cod) for dissolved or neutrally buoyant pollutants with very long biochemical half lives. This can be compared to a "cycling time" estimate by Csanady et al (1979) of about 1 year for this coastal current Gulf Stream gyre.

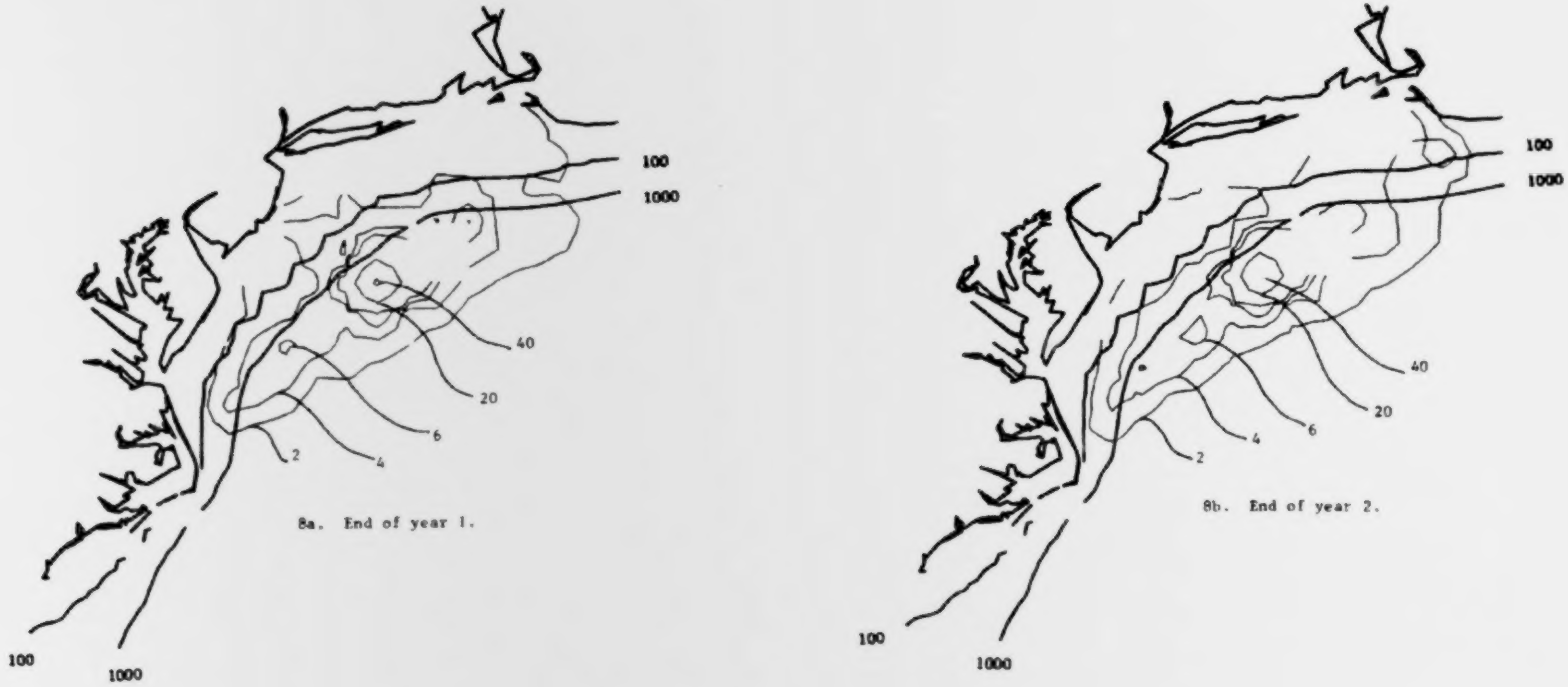
TABLE 1 MODEL INPUTS FOR PRELIMINARY TEST CASE

| Parameter | Value | Reference |
|---|---|---|
| Background PCB Concentration | 0.5 ng/l | O'Connor et al, 1983 |
| Suspended Solids Concentration | 0.3 mg/l | Chester, 1982 |
| PCB loading rate | 7.6 kg/day | Paul et al, 1983 |
| PCB (1254) Partition Coefficients: | | |
| - Octanol-water | $3 \times 10^6$ | Mackay, 1982 |
| - sediment-water | $1 \times 10^7$ | Nau-Ritter et al, 1982 |
| Particle settling rate: | | |
| 10% | 0.1 cm/sec | O'Conner et al, 1983 |
| 20% | 0.01 cm/sec | and |
| 20% | 0.001 cm/sec | Hawley, 1982 |
| 50% | Non-settling | |
| Dispersion Coefficient | $1 \times 10^7$ cm$^2$/sec | |
| Sediment Porosity | 80% | Schink et al., 1975 |

Figures 9a-9e show the modeled distribution of PCB on the sea floor as a function of time for that portion of the pollutant associated with particles settling at 0.01 cm/sec. Figures 10a-e show the same information for PCB scavenged by particles settling at 0.1 cm/sec. Particulates settling at lower velocities (Table 1) contribute negligibly to the buildup in the sediments. Figure 9 and 10 show that the pollutant loading to the sediments is relatively linear in time, since the modeled dumping rate is constant, the hydrodynamic transport field is steady, and the pollutant decay rate has been set to zero. Approximately 16% of the total PCBs dumped at DWD-106 will reach the seafloor on the continental shelf or shelf break. The bi-modal distributions of PCB in the sediments (Figures 9 and 10) result from reduced horizontal velocities in the shear zone between the coastal currents and the Gulf Stream (Figures 3 and 4).

Figure 11 shows the functional representation of the benthic loading within one grid cell of the transport model. Note that the unloading portion of the curve, from year 6 to year 30, is largely hypothetical, and assumes gradual desorption at the water/sediment interface. Figures 12a-12c show the computed distributions of PCB in the sediments through time from the beginning of the dumping policy until its cessation 6 years later. The rage of diffusion/bioturbation coefficients used here is taken from O'Connor et al. (1983) and Duursma and Smies (1982).

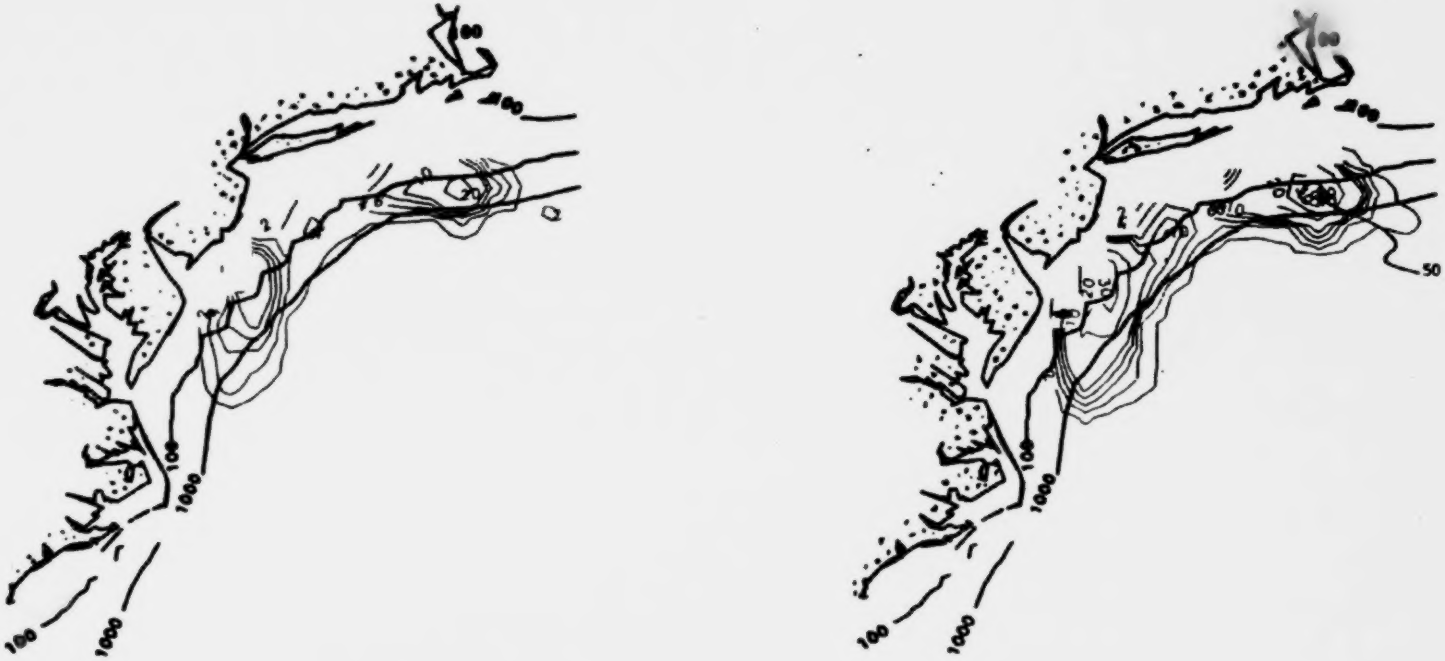
The importance of the bioturbation effect is further apparent from Figure 13, which shows the time histories of the concentration at a depth of 5 cm into the sediment for different values of the parameter D. It is clear that regardless of the bioturbation rate, the sediments will act as a very long term source for pollutants with low decay rates.


100
1000
40
20
6
4
2
100
1000
100
1000
40
20
6
4
2
100
1000

8a. End of year 1.

8b. End of year 2.

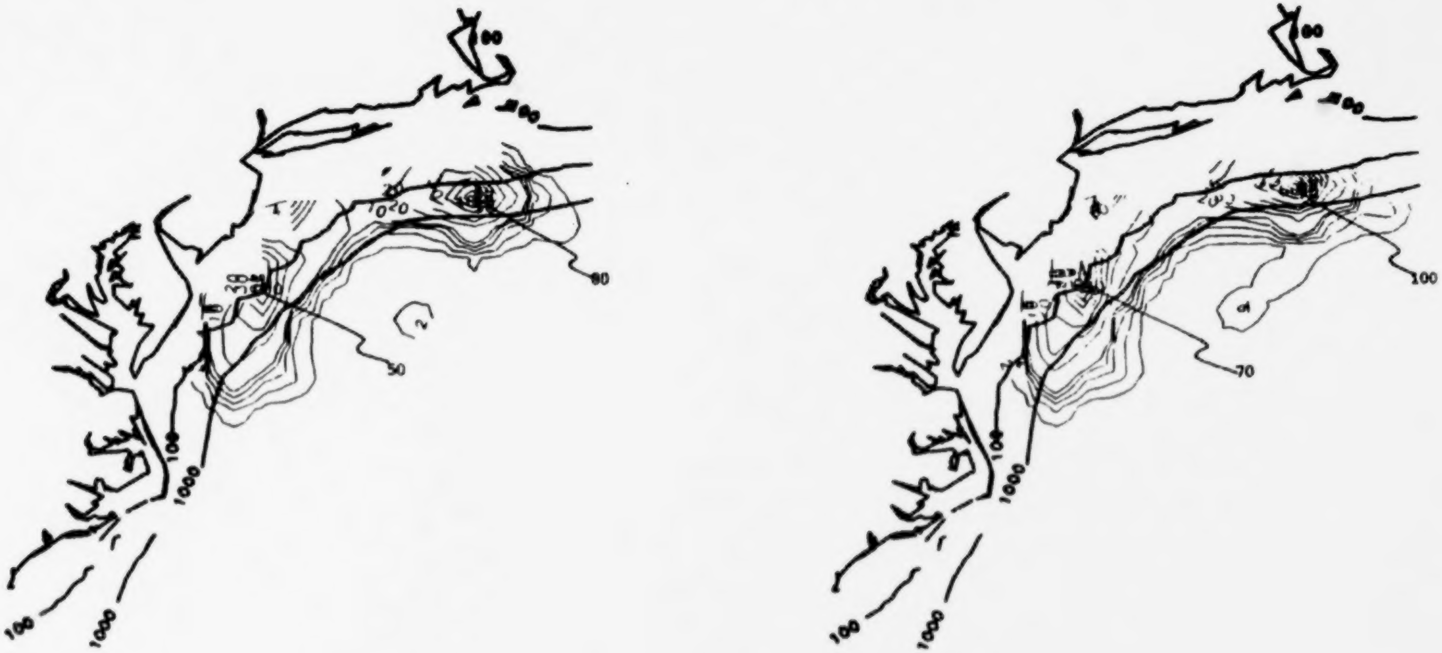
FIGURE 8 DISSOLVED PLUS ADSORBED PCB CONCENTRATIONS AVERAGED OVER THE TOP 100 METERS OF THE WATER COLUMN AT THE END OF SIMULATION YEARS 1 AND 2. (ng/m$^3$)



9a. End of year one.

9b. End of year two.

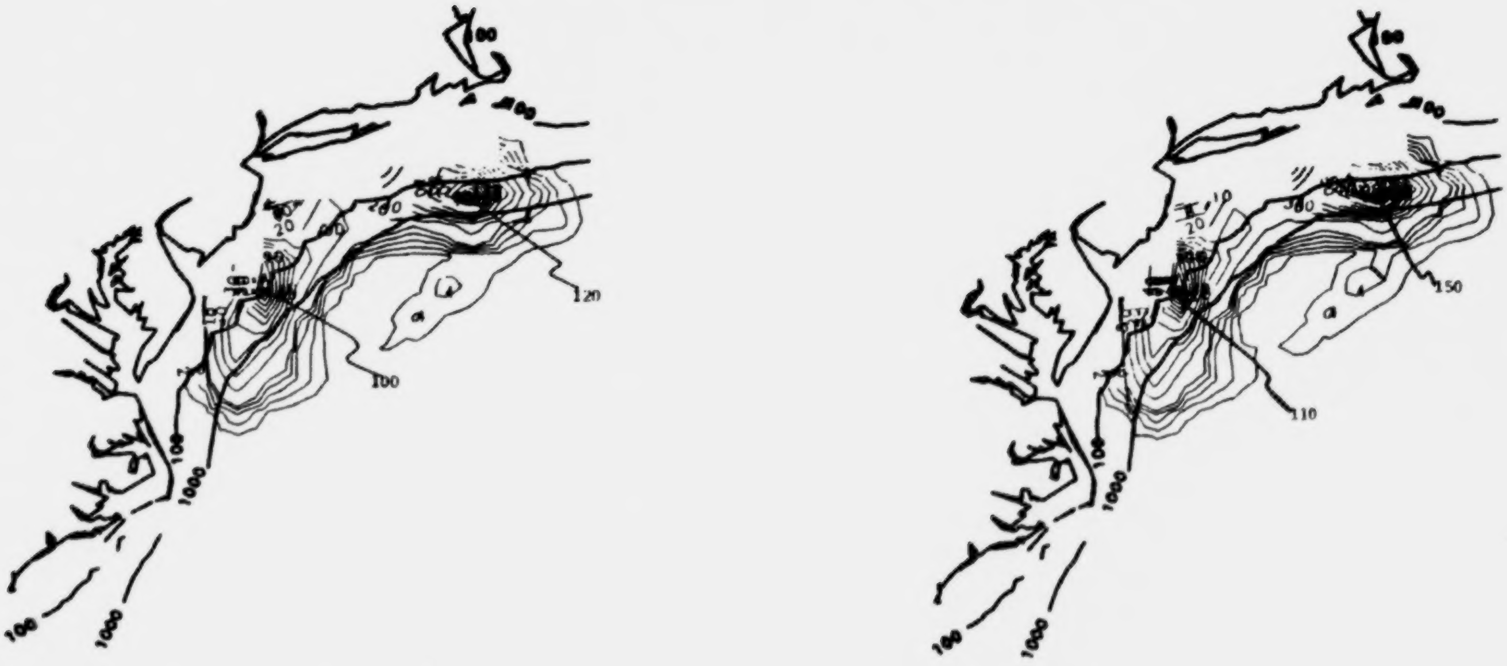
FIGURES 9A AND 9B MODELED SEA FLOOR DISTRIBUTIONS OF PCB (ug/m$^2$) FROM SEWAGE SLUDGE DUMPING AT THE 106 SITE DUE TO PARTICULATE SCAVENGING AND SETTLING AT 0.01 CM/SEC. TOTAL SEDIMENT CONCENTRATIONS ARE FOUND BY ADDING CONCURRENT FIGURES RESULTING FROM BOTH SETTLING SEDIMENT TYPES USED (e.g. superposition of Figures 9a and 10a)



9c. End of year three.

9d. End of year four.

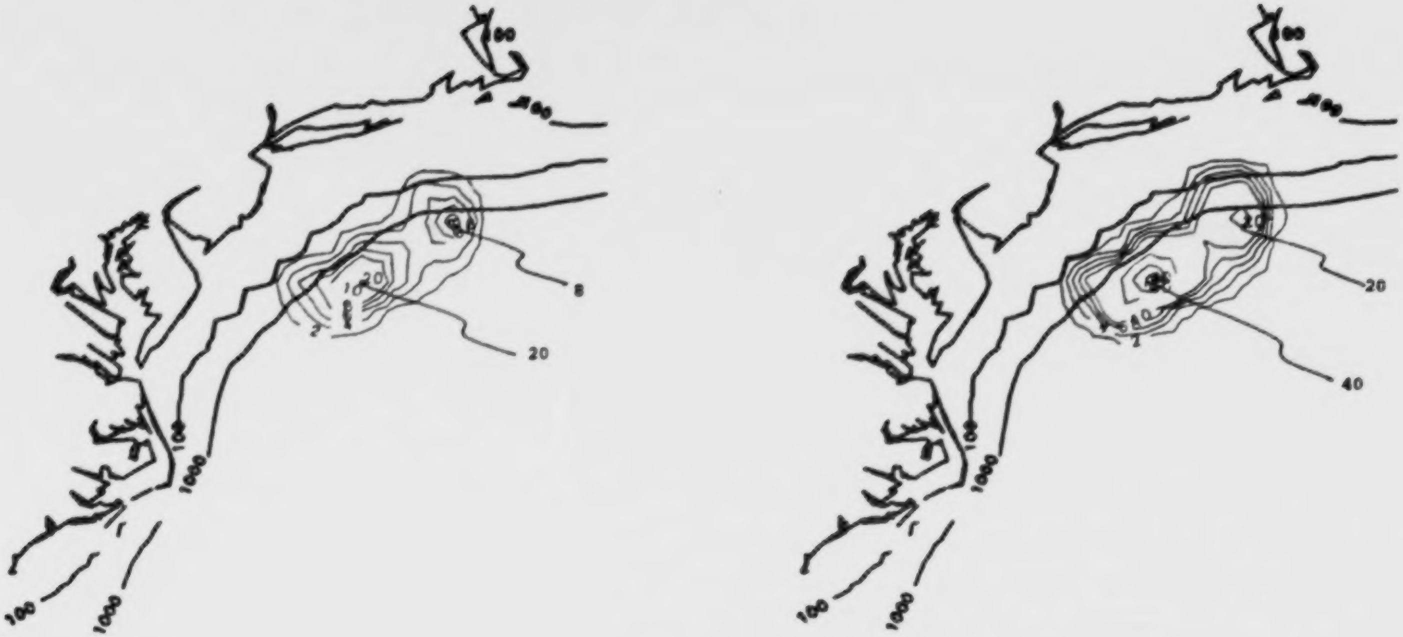
FIGURES 9C AND 9D MODELED SEA FLOOR DISTRIBUTIONS OF PCB (ug/m$^2$) FROM SEWAGE SLUDGE DUMPING AT THE 106 SITE DUE TO PARTICULATE SCAVENGING AND SETTLING AT 0.01 CM/SEC. TOTAL SEDIMENT CONCENTRATIONS ARE FOUND BY ADDING CONCURRENT FIGURES RESULTING FROM BOTH SETTLING SEDIMENT TYPES USED (e.g. superposition of Figures 9a and 10a)


100
20
200
120
100
100
1000
100
1000
20
300
150
110
100
1000
100
1000

9e. End of year five.

9f. End of year six.

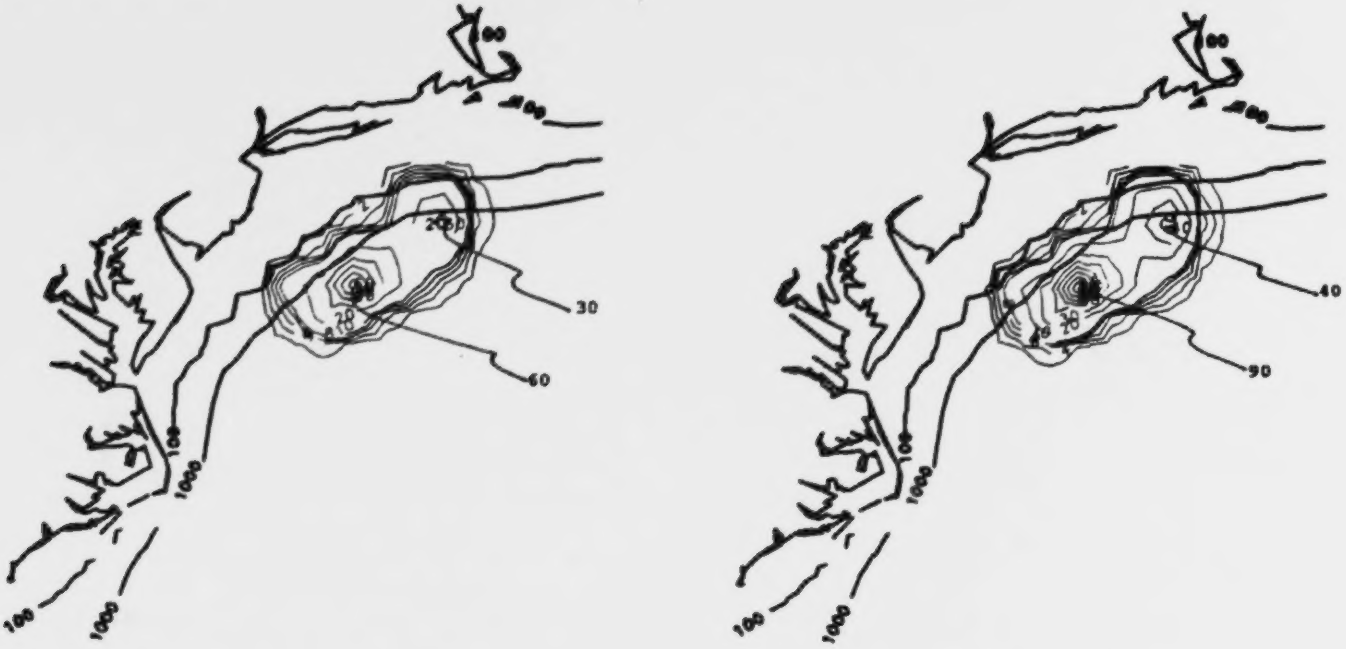
FIGURES 9E AND 9F MODELED SEA FLOOR DISTRIBUTIONS OF PCB (ug/m$^2$) FROM SEWAGE SLUDGE DUMPING AT THE 106 SITE DUE TO PARTICULATE SCAVENGING AND SETTLING AT 0.01 CM/SEC. TOTAL SEDIMENT CONCENTRATIONS ARE FOUND BY ADDING CONCURRENT FIGURES RESULTING FROM BOTH SETTLING SEDIMENT TYPES USED (e.g. superposition of Figures 9a and 10a)


100
200
8
20
10
20
2
100
1000
100
1000
100
200
20
40
2
100
1000
100
1000

10a. End of year one.

10b. End of year two.

FIGURES 10A AND 10F MODELED SEA FLOOR PCB DISTRIBUTION (ug/m$^2$) ASSOCIATED WITH PARTICLES SETTLING AT 0.1 CM/SEC. THE DISTRIBUTION OF SETTLING VELOCITIES FOR ALL PARTICLES IS GIVEN IN TABLE 1

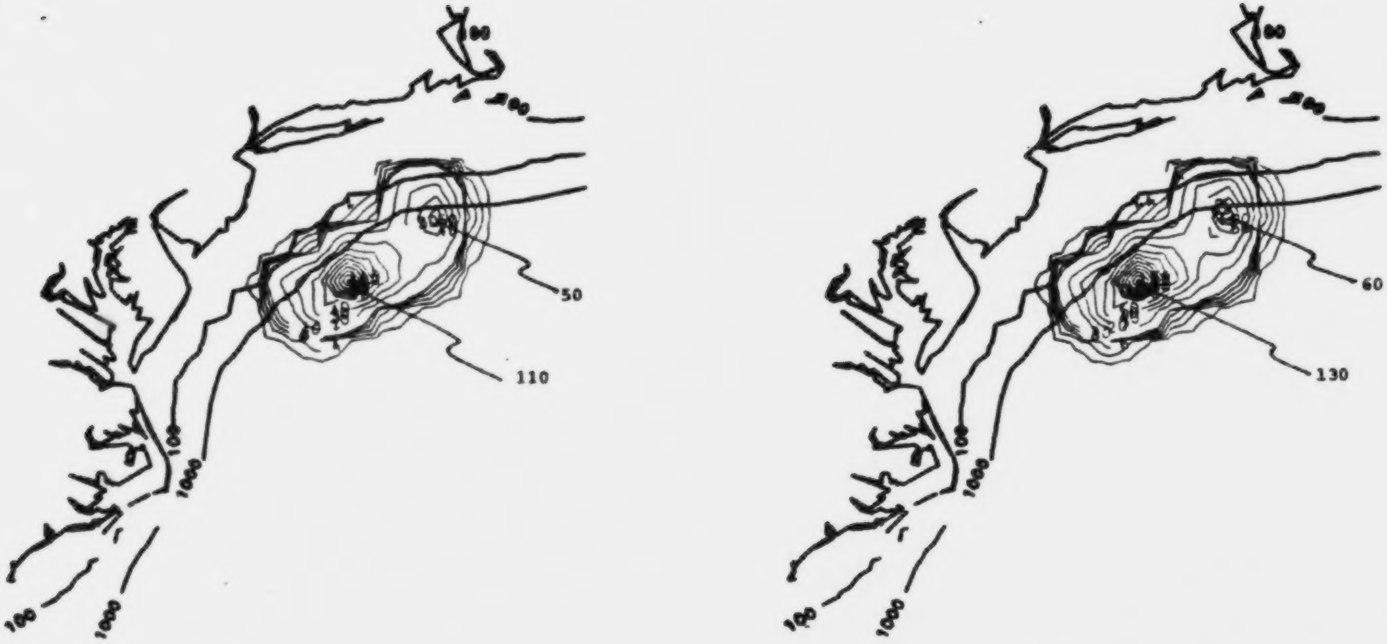


100
1000
200
30
60
40
90
20
10

10c. End of year three.

10d. End of year four.

FIGURES 10C AND 10D MODELED SEA FLOOR PCB DISTRIBUTION (ug/m$^2$) ASSOCIATED WITH PARTICLES SETTLING AT 0.1 CM/SEC. THE DISTRIBUTION OF SETTLING VELOCITIES FOR ALL PARTICLES IS GIVEN IN TABLE 1


80
200
50
110
100
1000
100
1000
80
200
60
130
100
1000
100
1000

10e. End of year five.

10f. End of year six.

FIGURES 10E AND 10F MODELED SEA FLOOR PCB DISTRIBUTION (ug/m$^2$) ASSOCIATED WITH PARTICLES SETTLING AT 0.1 CM/SEC. THE DISTRIBUTION OF SETTLING VELOCITIES FOR ALL PARTICLES IS GIVEN IN TABLE 1

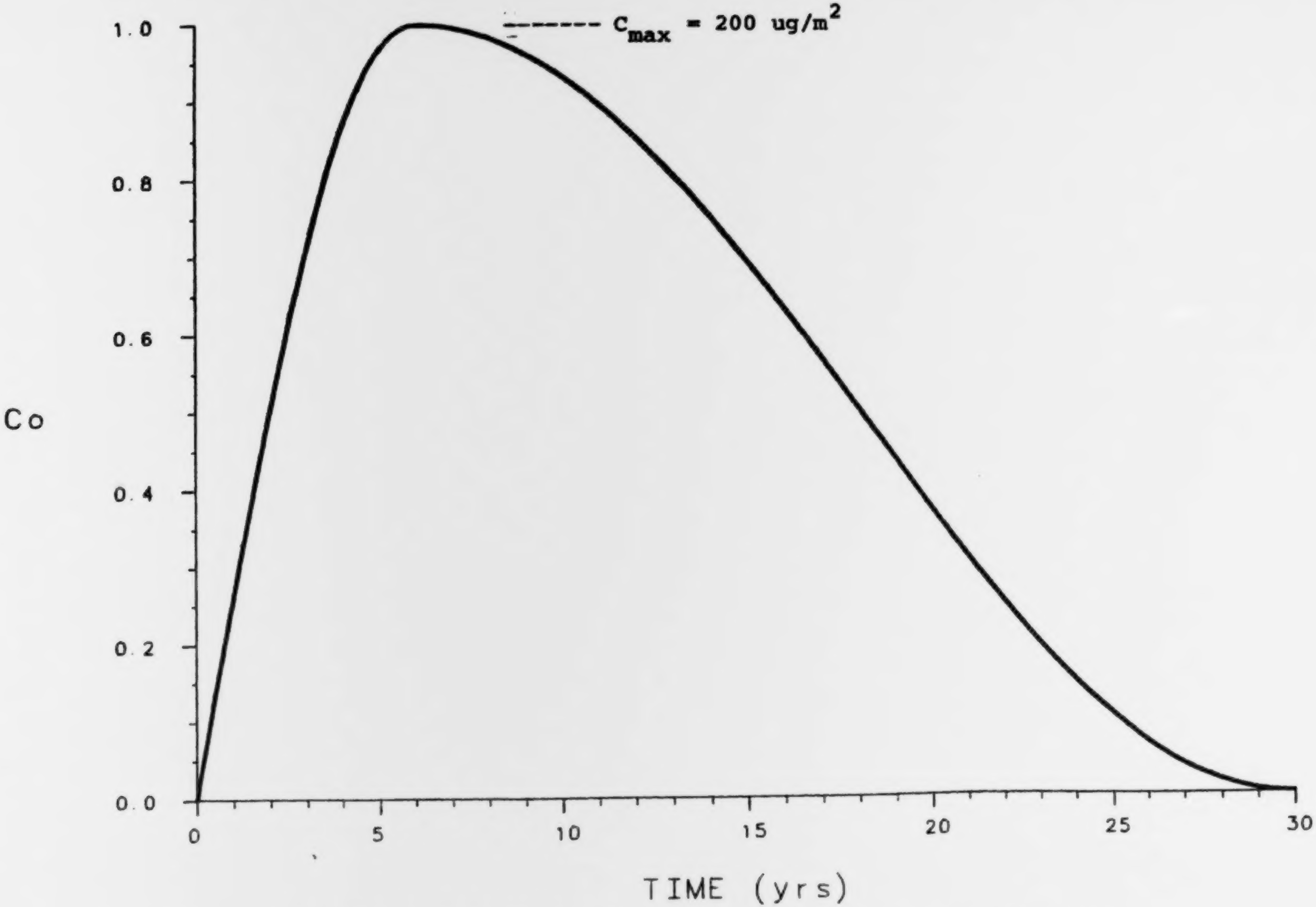


FIGURE 11 MODELED LOADING OF PCB AS A FUNCTION OF TIME AT THE SEDIMENT/WATER INTERFACE. THE LOADING IS INFERRED FROM THE TRANSPORT MODEL. THE UNLOADING IS HYPOTHETICAL, REFLECTING ESTIMATED REMOVAL THROUGH DISSOLUTION OF PCB AT THE INTERFACE

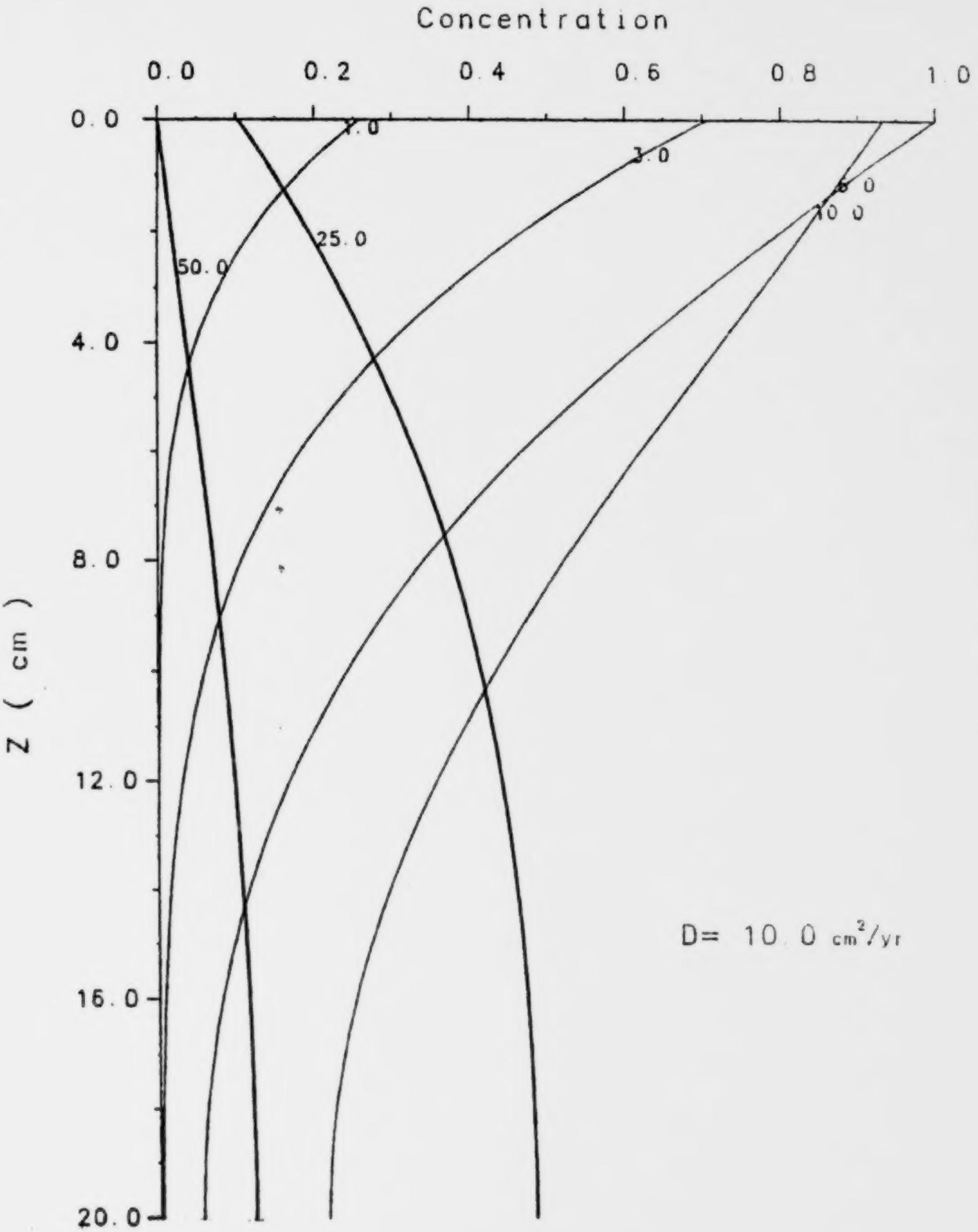


FIGURE 12A MODELED TIME HISTORY FOR PCB CONCENTRATIONS IN THE SEDIMENTS FOR 3 VALUES OF THE INTRA-SEDIMENT DISPERSION/BIOTURBATION COEFFICIENT. THE CURVES ARE LABELED IN YEARS AFTER START. THE INTERFACE VALUE IS GIVEN AS A FUNCTION OF TIME IN FIGURE 11, AND IS THE FRACTIONAL VALUE OF THE MAXIMUM CONCENTRATION OF 200 ug/m$^2$ PREDICTED BY THE POLLUTANT TRANSPORT AND FATE MODEL

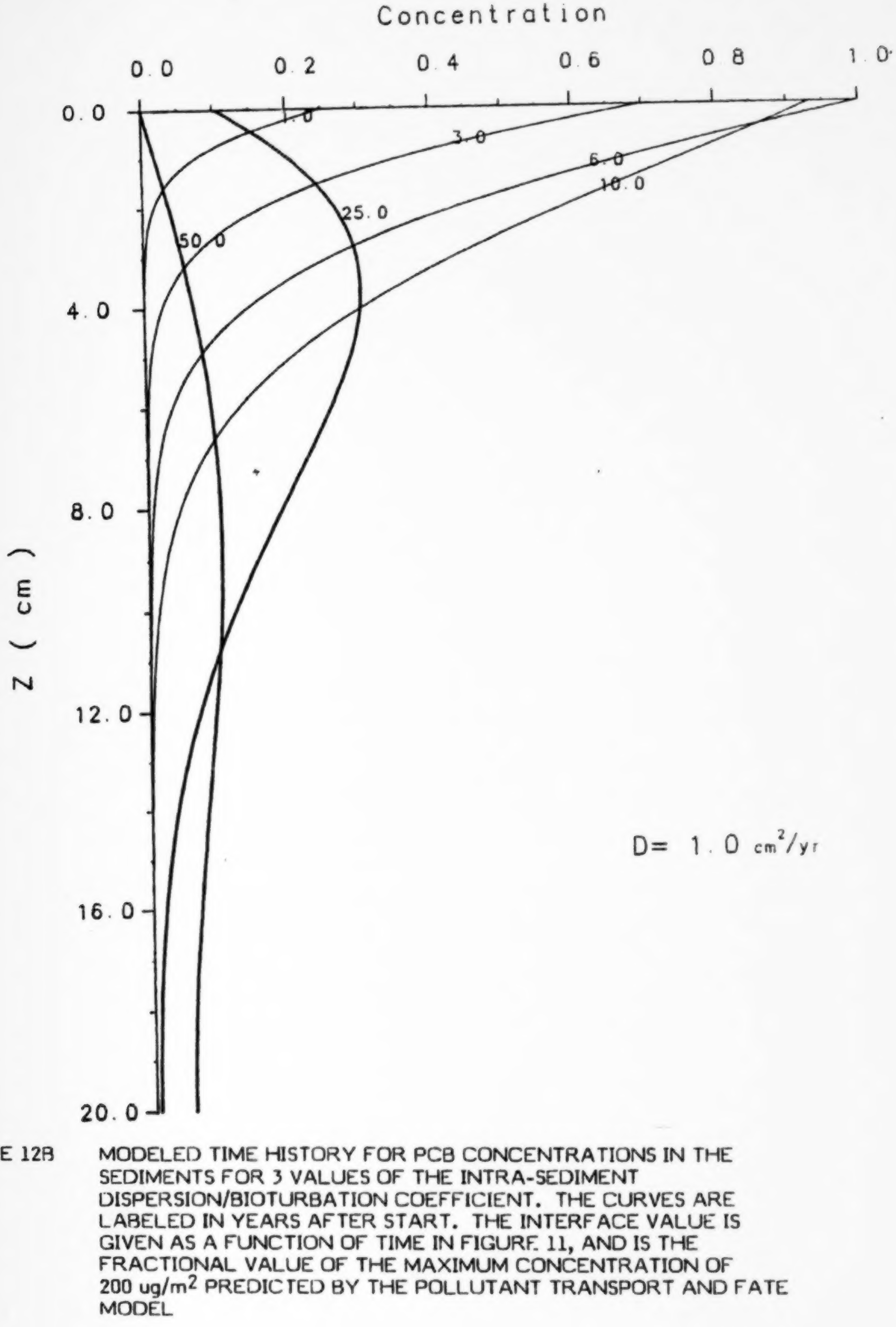


FIGURE 12B MODELED TIME HISTORY FOR PCB CONCENTRATIONS IN THE SEDIMENTS FOR 3 VALUES OF THE INTRA-SEDIMENT DISPERSION/BIOTURBATION COEFFICIENT. THE CURVES ARE LABELED IN YEARS AFTER START. THE INTERFACE VALUE IS GIVEN AS A FUNCTION OF TIME IN FIGURE 11, AND IS THE FRACTIONAL VALUE OF THE MAXIMUM CONCENTRATION OF 200 ug/$m^2$ PREDICTED BY THE POLLUTANT TRANSPORT AND FATE MODEL

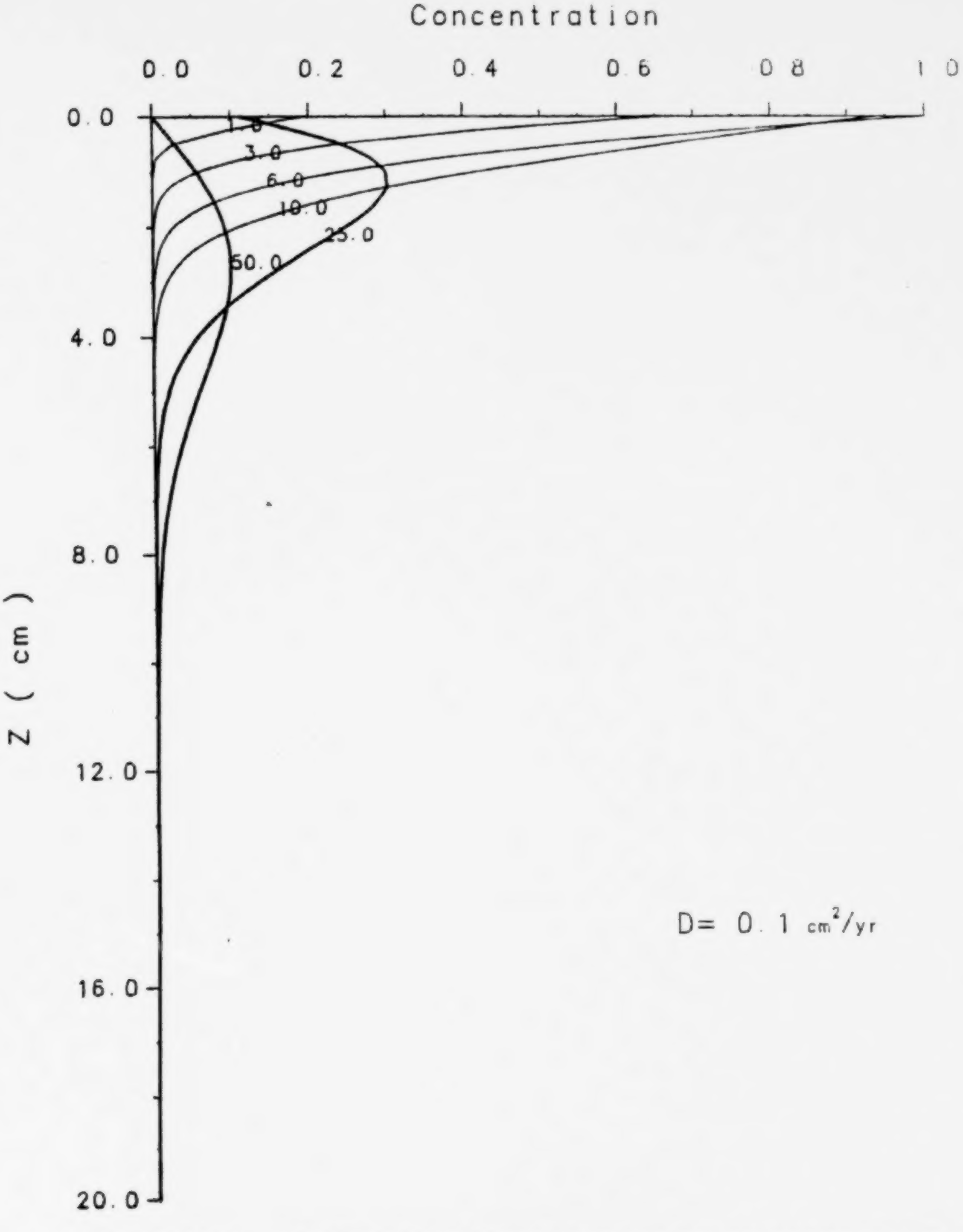


FIGURE 12C MODELED TIME HISTORY FOR PCB CONCENTRATIONS IN THE SEDIMENTS FOR 3 VALUES OF THE INTRA-SEDIMENT DISPERSION/BIOTURBATION COEFFICIENT. THE CURVES ARE LABELED IN YEARS AFTER START. THE INTERFACE VALUE IS GIVEN AS A FUNCTION OF TIME IN FIGURE 11, AND IS THE FRACTIONAL VALUE OF THE MAXIMUM CONCENTRATION OF 200 ug/m$^2$ PREDICTED BY THE POLLUTANT TRANSPORT AND FATE MODEL

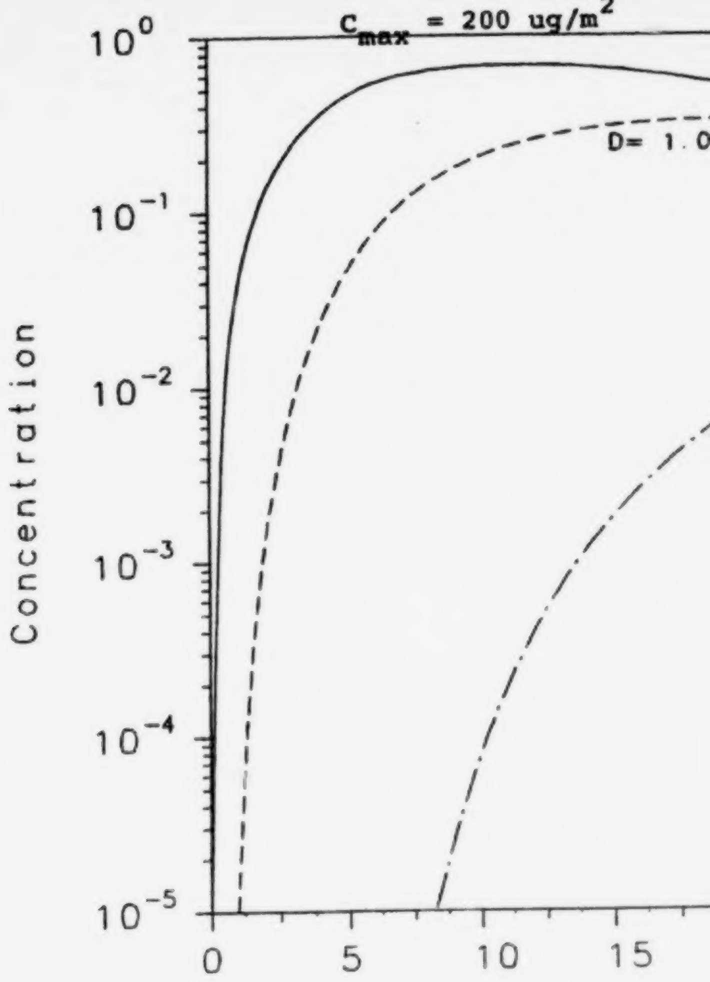
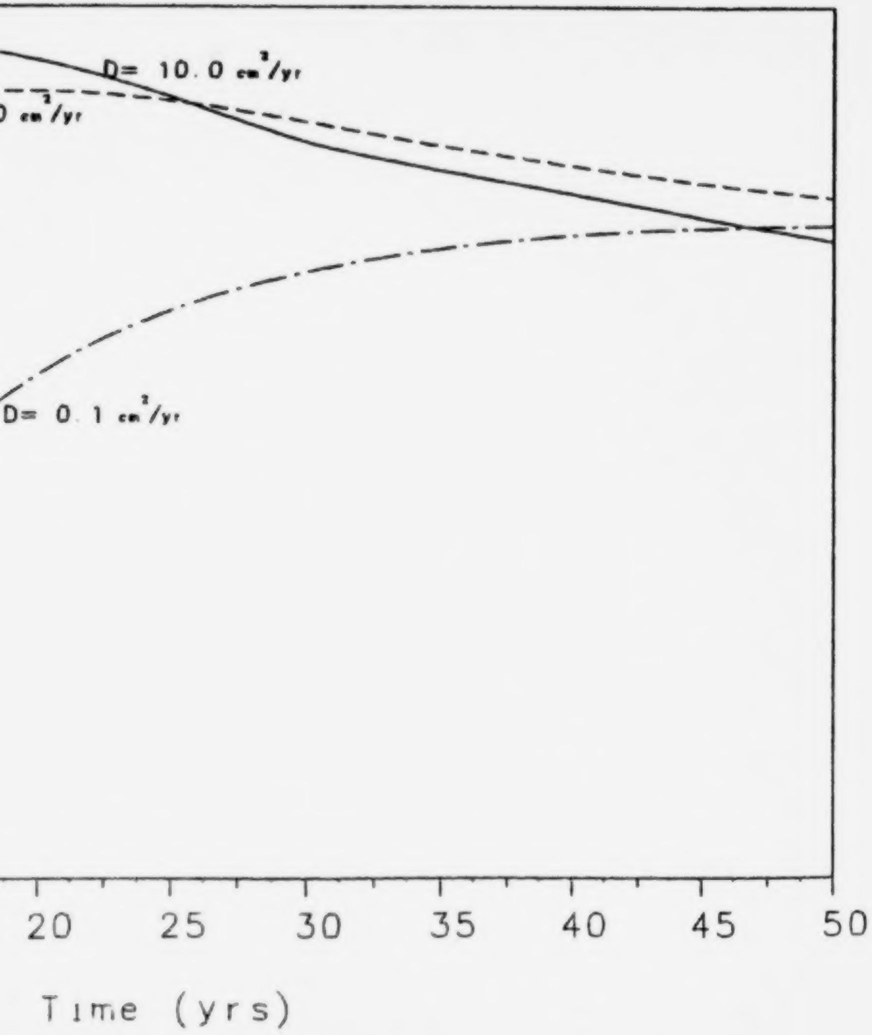


FIGURE 13 MODELED TIME HISTORY OF CONC
AT A DEPTH OF 5 CM BELOW THE
OF THE DIFFUSION/BIOTURBATION



CENTRATION, AS A FRACTION OF $C_{MAX}$ = 200 ug/m$^2$,
SEDIMENT/WATER INTER-$^{MAX}$ FACE FOR 3 VALUES
COEFFICIENT

Figure 14 shows the modeled bioaccumulation and depuration histories in the various trophic compartments of Figure 5. Because the large scale water column concentration changes predicted by the model are well below present background levels of 0.5 ng/kg (Table 1), changes in PCB concentrations at the plankton, zooplankton, and small pelagics levels (curves 1, 2, and 5 respectively) are negligibly affected.

The concentration levels in the benthic fauna respond directly to increases in sediment concentrations. Curve 3 in Figure 14 shows the modeled trace of this response. The demersal and large pelagic finfish (curves 4 and 6 respectively), reflect the food chain responses to this increased benthic loading. For comparison, Boehm and Hirtzer (1982), report PCB concentrations in cod to range from 0.2 to 5 ppb wet weight (assuming a factor of 0.2 for conversion from dry weight to wet weight). If the present average is 1 ppb, the model system estimates an approximate doubling of the PCB concentrations in cod and similar species in the area. Note that this implication remains relatively speculative at this time, given the preliminary state of the model system.

## CONCLUSION

We have presented the present status of an ocean dumping impact assessment model system. The intent of system design has been to include the simplest representations which are believed to adequately reflect the dynamics of important governing processes. The processes included are:

(1) spatially variable hydrodynamic transport, with the potential for vertical variations;
(2) particulate/dissolved phase partitioning in the water column and sediments;
(3) pollutant sequestering to the sediments via particulate adsorption and settling;
(4) bioaccumulation via absorption and food web dynamics;
(5) biomass (and yield) reductions through growth or reproductive rate reductions or mortality rate increases.

A preliminary test of the system shows reasonable results relative to field measurements, although considerable work remains before model reliability can be established in the context of parameter uncertainty.

The model suggests that about 16% of the PCB input with sewage sludge at DWD-106 will reach the sediments within 300 km of the site. The remainder, whether particle-adsorbed or dissolved, will be flushed from the system within 1.5 to 2 years of its introduction.

## DISCLAIMER

Although the work reported here has been funded wholly or in part by the United States Environmental Protection Agency through Contract No. 68-01-6621, it has not been reviewed by Agency personnel, does not represent Agency policy views, decisions, or viewpoints, and no official endorsement should be inferred.

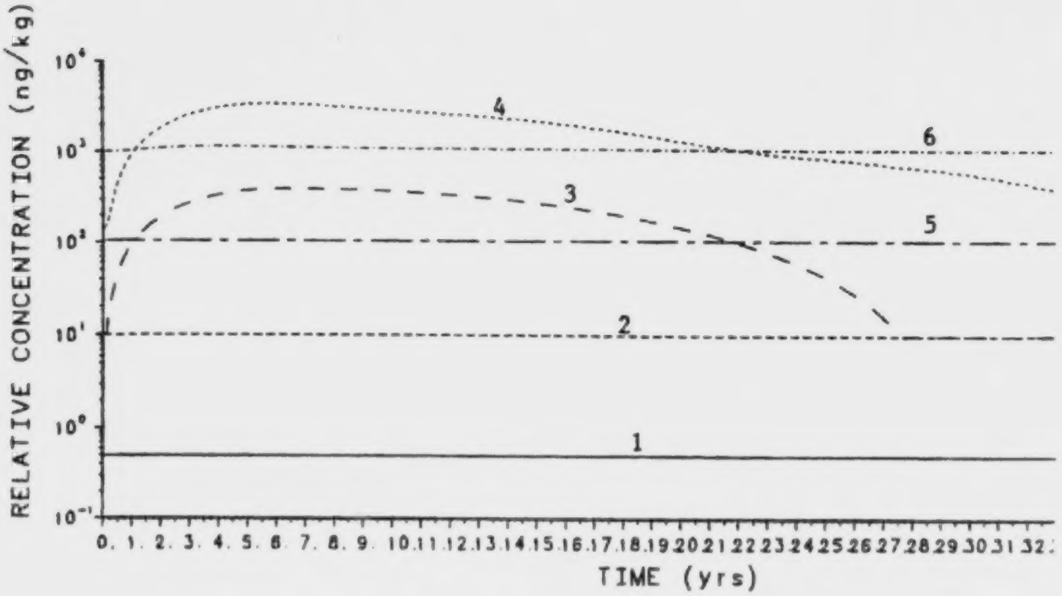


FIGURE 14 DYNAMIC TRACE OF PCB CONCENTRATIONS IN 6 TROPHIC COMPARTMENTS. (1-PHYTOPLANKTON; 2-ZOOPLANKTON; 3-BENTHOS; 4-DEMERSAL FINFISH; 5-SMALL PELAGICS; 6-LARGE PELAGICS)

## ACKNOWLEDGEMENTS

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# COMPARISON OF SPECIES SENSITIVITIES TO TOXICANTS USING NATIONAL WATER QUALITY CRITERIA

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LOZANO, S. 1985. Comparison of species sensitivities to toxicants using National Water Quality Criteria. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 125-126.

Of the billion kilograms of chemicals that are manufactured every year, a significant amount enters our waterways. The objectives of our research were formulated as a response to the needs of industry and regulatory agencies to evaluate the potential toxicity of these chemicals to aquatic species. The available information on toxicity exists only for a small fraction of the 45 000 chemicals commercially manufactured. In order to develop predictive capabilities to estimate the toxicity of aquatic pollutants, considerable effort must be focused on developing a reliable data base and a predictive assessment methodology. The accuracy of the prediction will depend on the quality of information on biological responses, experimental conditions, and methods for data analysis. The systematic, computerized compilation of aquatic toxicity data in the AQUatic Information REtrieval (AQUIRE) system provides sufficient information on single compounds to allow comparison between organisms, chemicals and test endpoints.

The initial steps necessary to compare species sensitivities to toxicants include data categorization and development of standardized analytical methods. Acute toxicity data from AQUIRE were grouped into several major categories such as metals, pesticides, inorganic anions, alcohol, chlorophenols, ethers and chlorinated alkanes. Once chemicals were grouped, comparison of species specific acute toxicity was accomplished by calculating U.S. National Water Quality Criteria. This allowed a comparison of individual species mean acute values (SMAVs) with a non-biased standard, the fifth percentile of a set of SMAVs. The relative sensitivity of individual, or groups of similar, species could then be compared across similar chemicals without making adjustments for sample size or data variability. This is a necessary step for the development of predictive estimates of chemical toxicity.

LOZANO, S. 1985. Comparison of species sensitivities to toxicants using National Water Quality Criteria. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 125-126.

Sur le milliard de kilogrammes de produits chimiques qui sont fabriqués chaque année, une quantité importante pénètre dans nos voies d'eau. Les objectifs de notre recherche ont été formulés en réponse aux besoins de l'industrie et des organismes de réglementation, qui nous demandent d'évaluer la toxicité potentielle de ces produits chimiques pour les espèces aquatiques. L'information dont nous disposons sur cette toxicité n'existe que pour une faible fraction des 45 000 produits chimiques fabriqués et commercialisés. Afin de mettre au point les moyens d'estimer à l'avance la toxicité des

polluants aquatiques, des efforts considérables doivent être déployés en vue d'établir une base de données fiables, ainsi qu'une méthodologie d'établissement de prévisions. La précision des prévisions dépendra de la qualité de l'information recueillie sur les réactions biologiques, les conditions expérimentales et les méthodes d'analyse des données. La compilation systématique et informatisée des données sur la toxicité aquatique grâce au système AQUIRE (AQUatic Information REtrieval = recherche documentaire dans les domaines aquatiques) fournit une information suffisante concernant les produits isolés et permettant la comparaison entre organismes, produits chimiques et points limites des tests.

Parmi les étapes initiales que nécessite la comparaison entre les sensibilités individuelles des espèces aux polluants, citons la catégorisation des données et le développement de méthodes analytiques normalisées. Les données concernant la toxicité aigue, fournies par le système AQUIRE, ont été regroupées en plusieurs catégories majeures: métaux, pesticides, anions inorganiques, alcool, chlorophénols, éthers et alcanes chlorés. Une fois les produits chimiques groupés, la comparaison entre les toxicités aigues pour les diverses espèces a été effectuée par calcul des critères nationaux américains relatifs à la qualité des eaux. De cette façon, on a obtenu une comparaison des valeurs moyennes aigues des diverses espèces (SMAV) et une norme non biaisée, le cinquième centile d'un jeu de SMAV. La sensibilité relative d'espèces particulières ou de groupes d'espèces similaires peut alors être comparée par rapport à des produits chimiques similaires sans qu'on ait à faire d'ajustement pour la dimension des échantillons ou la variabilité des données. Cette étape est nécessaire au développement des prévisions de la toxicité chimique.

# AQUIRE: AQUATIC TOXICITY INFORMATION RETRIEVAL

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PILLI, A. 1985. Aquire: Aquatic Toxicity Information Retrieval. Can. Tech. Rep. Aquat. Sci. 1368: pp. 127-128.

The AQUIRE data base was established to provide a comprehensive, systematic, computerized compilation of aquatic toxicity data.

Papers published world-wide on toxicity of chemicals to aquatic organisms are collected and reviewed for AQUIRE. Emphasis has been on papers published between 1972-1981, and only primary references' data are included. Selected information from and results of toxicity tests are extracted and added to the data base; acute, sublethal, and bioaccumulation effects are entered. Toxicity tests with freshwater and saltwater organisms (except bacteria) are included, for any chemical except complex effluents or oils. Combined pollutant toxicity tests are not included. A unique characteristic of AQUIRE is the incorporation of a quality review code. Depending on the methodology documentation and caliber of test methods, encoded data from tests are assigned a rating for reliability of results.

Data stored in computer files can be easily retrieved and outputted onto video terminals, line printers or magnetic tapes. A straight dump of the output can provide information on toxicity data, along with reference citations. Further sorting programs provide the capability to establish quantitative relationships between toxicity and different test conditions.

AQUIRE now has on computer file 37 000 data entries for 2 000 organisms. Toxicity data for 2 000 chemicals have been encoded for AQUIRE, which includes a "quality of test data" rating, and all entries have been subjected to established quality assurance procedures. Of approximately 5 000 publications acquired, 3 750 have been reviewed for inclusion in AQUIRE. Of the publications not reviewed for AQUIRE approximately 400 have no codeable toxicity data, 400 are foreign papers needing translation, and the remaining 200 are combined pollutant, oil, complex effluent papers which are held for future use.

PILLI, A. 1985. Aquire: Aquatic Toxicity Information Retrieval. Can. Tech. Rep. Aquat. Sci. 1368: pp. 127-128.

La base de données AQUIRE a été établie afin de fournir une compilation exhaustive, systématique et informatisée des donées sur la toxicité aquatique.

Les communications publiées à l'échelle mondiale concernant la toxicité des produits chimiques pour les organismes aquatiques sont rassemblées et révisées pour AQUIRE. L'intérêt s'est porté essentiellement sur les publications parues entre 1972 et 1981, et seules figurent les données de référence primaire. Les informations choisies à partir des tests de toxicité, ainsi que les résultats de ces tests, sont extraites et portées dans la base de données; toxicité aigue, toxicité sublétale, et effets de la bio-accumulation sont aussi entrés dans la base de données. On y trouve également les tests de toxicité pratiqués sur des organismes d'eau douce et d'eau salée (à l'exception des bactéries), pour tous les produits chimiques, excepté les effluents complexes et les pétroles. N'y figurent pas les tests de toxicité portant sur les polluants associés. Une caractéristique exceptionnelle d'AQUIRE est l'incorporation d'un code de qualité. En fonction de la documentation sur la méthodologie et de la valeur des méthodes d'essai, les données codées provenant des tests reçoivent une évaluation correspondant à la fiabilité des résultats.

Les données classées dans les mémoires de l'ordinateur sont facilement accessibles et peuvent être présentées sur terminal vidéo, imprimante ou bande magnétique. Un simple vidage des résultats permet d'obtenir l'information sur les données de toxicité, ainsi que les citations de référence. Des programmes de tri donnent la possibilité d'établir des relations quantitatives entre la toxicité et différentes conditions d'essai.

AQUIRE a actuellement en mémoire 37 000 données sur 2 000 organismes. Des données de toxicité portant sur 2 000 produits chimiques ont été codées pour AQUIRE, comportant une évaluation de la qualité des données des tests, et toutes les entrées ont été soumises à des procédures établies d'assurance de la qualité. Sur environ 5 000 publications recueillies, 3 750 ont été révisées en vue de leur inclusion dans AQUIRE. Des publications non revues pour AQUIRE, environ 400 n'ont aucune donnée de toxicité codable, 400 autres sont des publications étrangères nécessitant une traduction, et les 200 restantes sont des publications touchant des polluants associés, le pétrole, ou des effluents complexes (elles sont conservées en vue d'une utilisation future).

# ALTERNATIVE END POINTS AND CALCULATION PROCEDURES TO ANALYSIS OF BIOASSAY DATA

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SHIRAZI, M.A. 1985. Alternative end points and calculation procedures to analysis of bioassay data. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 129-130.

EC50, LC50..., etc condense bioassay data into biologically meaningful end point numbers (scalers). They are widely used and often simple to calculate. They are probably most useful for comparing results of bioassays or similar organisms. This paper explores the utility of alternative scalers to EC50 that can better integrate bioassay data when diverse organisms and mixes of chemicals are used.

When bioassay data exhibit unpredictable fluctuations, the calculation of EC50, LC50..., becomes difficult, often causing researchers to question the results and perhaps to discard or conveniently smooth the outliers they do use. There is no universally satisfactory method to handle the data. This paper presents a robust procedure for calculating EC50 or other alternative scalers to include all data. The procedure is capable of handling every course data with little manipulation.

The procedure developed in this paper is based on the use of a centroid to calculate the central tendency of the integrated area under the dose/response curve. It considers the effective dose and response ranges and the sensitivity of dose response relationship. The paper explores combining data from diverse experiments on chemicals and organisms by using one or more of these scalers as integrators. A large data base on root germination demonstrates the potential utility of the approach.

SHIRAZI, M.A. 1985. Alternative end points and calculation procedures to analysis of bioassay data. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 129-130.

Les CE50, CL50, etc. permettent de condenser les données de bioessais en des valeurs de référence valables (points de référence). Ces valeurs sont largement utilisées, et sont souvent simples à calculer. Leur utilité fondamentale est probablement de servir à comparer les résultats de bio-essais sur des organismes similaires. Notre publication a pour objet d'analyser l'utilité de points de référence destinés à remplacer la CE50 afin de mieux intégrer les données de bio-essais lorsqu'elles portent sur des organismes divers et des mélanges de produits chimiques.

Lorsque les données de bio-essais présentent des fluctuations imprévisibles, le calcul de la CE50, CL50, etc. devient difficile et conduit souvent les chercheurs à mettre leurs résultats en doute, et même parfois à laisser de côté les valeurs déviantes ou encore à adoucir les courbes obtenues. Pour traiter les données, il n'existe pas de méthode qui soit satisfaisante à tous les points de vue. Dans la présente communication, nous présentons

une solide méthode de calcul applicable à la CE50 ou à d'autres points de référence et permettant d'inclure toutes les données. Enfin, cette méthode permet de traiter des données passablement brutes avec peu de manipulation.

La méthode exposée ici est basée sur l'utilisation d'un centre de gravité permettant de calculer la tendance centrale de la surface intégrée sous la courbe dose-effet. Elle tient compte des plages de dose et d'effets efficaces ainsi que de la sensibilité de la relation dose-effet. On y étudie le moyen de combiner des données provenant d'expériences diverses sur des produits chimiques et des organismes en utilisant une ou plusieurs de ces points de référence comme intégrateur. Pour montrer l'utilité de cette méthode, nous présentons une vaste base de données concernant la germination radiculaire.

# PROBLEMS OF INTERPRETING SCIENTIFIC DATA FOR RESOURCE MANAGEMENT

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WHITE, H.H. and G. PETRAZZVOLO. 1985. Problems of interpreting scientific data for resource management. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 131-132.

This paper deals with a concern that current marine pollution assessment strategies are disjointed and piecemeal. No one provides the framework for assembling these pieces into a quantitative whole. This paper describes the details of such a holistic framework, and argues for its adoption.

The proper function of science in environmental assessments is to trace contaminants from their release by man to their ultimate effects on man's health, economy, food supply, recreation, and aesthetic sensitivities. The predictive output of the holistic model, effects on human uses of the oceans, is not a value that can be quantified precisely, no matter how tightly it is defined. There are two sources of error. First, there is the usual sampling error associated with the measurements that are entered into the model's equations. Second, there is the error associated with choice of simplifying assumptions and choice of coefficients when writing the model's equations. Most holistic pollution assessment models will include both kinds of error, rendering any estimates in the model's total predictive error very coarse.

National governments must take the lead in holistic assessments of pollution issues. An agency program whose mission is long-term environmental research is the most likely candidate to pioneer the concept. Since point source loading is much easier to characterize than non-point source loading, the first holistic effort should deal with an ocean disposal or ocean outfall problem.

The numerous and obvious benefits of the holistic approach to pollution assessment are discussed. The holistic strategy is not original with us.

WHITE, H.H. and G. PETRAZZVOLO. 1985. Problems of interpreting scientific data for resource management. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 131-132.

La présente publication traite du problème que posent actuellement les méthodes d'évaluation de la pollution marine, qui sont à la fois incohérentes et fragmentaires. Jusqu'ici, personne n'a fourni de cadre qui permette d'assembler ces détails en un tout quantitatif. Nous décrivons donc les détails d'un tel cadre holistique et tentons d'en encourager l'adoption.

Le principal objectif scientifique, dans les évaluations environnementales, est de retracer le parcours des contaminants depuis leur rejet par l'homme jusqu'à leurs effets ultimes sur sa santé, son économie, son alimentation, ses loisirs et ses valeurs esthétiques.

Les résultats prévisionnels du modèle holistique, les effets de l'utilisation que l'homme fait des océans, ne sont pas des valeurs quantifiables avec précision, quelle que soit la rigueur avec laquelle on peut les définir. Il existe deux sources d'erreur. La première est l'erreur habituelle d'échantillonnage associée aux mesures utilisées dans les équations du modèle. La deuxième est l'erreur associée au choix d'hypothèses de simplification et au choix des coefficients que l'on utilise au moment de construire les équations du modèle. Dans la plupart des modèles holistiques d'évaluation de la pollution, on retrouvera ces deux sortes d'erreurs qui rendront très grossière toute estimation de l'erreur prévisionnelle totale du modèle.

Les gouvernements nationaux doivent faire oeuvre de pionniers en évaluant de façon holistique les problèmes de pollution. Un programme gouvernemental dont la mission est la recherche environnementale à long terme serait probablement le meilleur moyen d'ouvrir le chemin. Une charge à source ponctuelle étant plus facile à caractériser qu'une charge à source non ponctuelle, le premier effort d'évaluation holistique devrait s'appliquer à un problème de rejet ou de déversement en mer.

Dans la présente publication, nous passons en revue les avantages nombreux et évidents que présente une méthode holistique d'évaluation de la pollution. Cette méthode ne nous est pas particulière.

# TOXICITY AND PH

G.F. Westlake, Chairman

# GENETIC CONTROL OF RESISTANCE TO LOW pH IN ALTLANTIC SALMON AT THE FAMILY AND STOCK LEVEL

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SCHOM, C.B. 1985. Genetic control of resistance to low pH in Atlantic salmon at the family and stock level. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 135-148.

Atlantic salmon were tested at two levels of genetic organization for genetic differences in survival time at low pH. The first level was within the stock. Here results unequivocally indicate that differences do exist between families and that these differences are hereditary.

The second level was at the stock level. Here results support the hypothesis that differences exist between stocks and that these differences may be accounted for by differences in pH in the native system.

In addition, arguments are presented to support the assumption that tests at lethal pH levels give rankings corresponding to those that would be made using chronic concentrations of hydrogen ion.

SCHOM, C.B. 1985. Genetic control of resistance to low pH in Atlantic salmon at the family and stock level. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 135-148.

Des saumons de l'Atlantique ont été testés à deux niveaux de l'organisation génétique, en vue d'apprécier les différences génétiques que présente leur temps de survie lorsqu'ils sont exposés à de faibles pH. Le premier niveau a été au sein même du stock. Dans ce cas, les résultats indiquent sans équivoque que des différences existent réellement entre les familles et que ces différences sont héréditaires.

Le deuxième niveau a été celui des stocks. Ici, les résultats viennent appuyer l'hypothèse que des différences existent entre stocks et que ces différences peuvent être mises sur le compte des différences de pH dans le système natal.

De plus, nous présentons des arguments destinés à soutenir l'hypothèse que des tests à des niveaux de pH létaux donnent des classements correspondant à ceux qui seraient faits en utilisant des concentrations chroniques d'ions hydrogènes.

## INTRODUCTION

Many river systems in the world show a decline in pH. This world-wide phenomenon, which is accompanied by either a reduction in number and/or elimination of fish species, has been reported for Swedish lakes (Almer, 1974), Norwegian lakes and rivers (Jensen and Snekvik, 1972 and Leivestad and Muniz, 1976) and North American systems (Haines, 1981; Johnson, 1982) - Canadian (Beamish and Harvey, 1972; Beamish, 1974 and 1976; Thompson et al., 1980 and Watt et al., 1983) and U.S. (Blake, 1981 and Rahel and Magneson, 1983) lakes and rivers. Once the pH falls below 5 to 5.5 in Swedish rivers, no Atlantic salmon recruitment occurs (Jensen and Snekvik, 1972), while the critical levels in Nova Scotia rivers appears to be between 4.7 and 5.0 (Watt et al., 1983).

Genetic variation in tolerance to low pH has been reported for Brown trout (Gjedrem, 1976), Brook trout (Swartz et al., 1978), Atlantic salmon (Schom and Davidson, 1982) and Yellow perch (Rahel, 1983). Rahel (1983) and Swartz et al. (1978) in addition, report strain or stock specific differences in response for Yellow perch and Brook trout respectively.

The purpose of this study was to investigate Atlantic salmon stock specific differences in resistance to low pH. The procedure was first to establish, over two generations, a set of lines that had a known relative resistance. Parr from these lines (families having same parents) were then used as a base line to compare and rank random samples of parr from different hatchery stock.

### Materials and Methods

### Family Trials

The family trials were run in consecutive years using two generation of males, plus two-year classes of males and females. In the first year, each female was crossed with two males, and in the second year, each female was mated with three males.

All females and one of the males in each cross in each year were wild anadromous salmon collected from the Big Salmon River. The other male in each cross in the first year, 1980, was a precocious parr selected at random from hatchery-reared Big Salmon River stock. The other two males per female in the second year, 1981, were precocious parr resulting from the first year crosses. The parr used in the second year were from families with a tested known response to low pH (unpublished data). All offspring in the family trials were pedigreed, that is, each cross was kept separate and the offspring then identified as to individual parents.

Each year-class was acid challenged three times (see below for details). The families were ranked based on mean survival time, and correlations (Snedecor and Cochran, 1968) were done on the rankings between the three trials in generation one and the mean survival time for the selected par offspring families in generation two.

The results from tests on generation two were corrected (see below for details) for survival time so that trial-to-trial differences were minimized. Thus, the means used to calculate the second generation two ranks were from the three tests on that generation.

## Strain Trials

Strain trials were done in a quarantine facility located at the University of New Brunswick in Saint John. The facility consisted of two recirculation units each containing 1 100 litres of water. Each unit supplied five, 1/2 meter test tanks. The replacement rate was 10% per hour. All supply water was dechlorinated, and all effluent was chlorinated (Brenegan and Schom, in preparation).

Five stock, 100 fish each, were brought to the quarantine facility. They were separated into two sets of 50 fish each and assigned at random one set to one tank in each unit. This was done a second time with four different stocks and one of the previously tested stocks (Table 1).

TABLE 1 STRAIN TRIAL - STOCK IDENTIFICATION AND DISTRIBUTION TO THE EXPERIMENTAL UNIT FOR TRIALS I AND II

Stock Identification

| Identification Number | Stock | Mean Length | River* pH | Hatch Year |
|---|---|---|---|---|
| 4 | Liscombe (Hatchery) | 8.3 | 4.8-5.1 | 1982 |
| 5 | Margaree (Wild) | 8.4 | 6+ | 1982 |
| 6 | Medway (Wild) | 12.9 | 4.9-5.8 | 1982 |
| 7 | East River (Kelts) | 8.0 | 4.8-5.4 | 1982 |
| 8 | Le Have (Hatchery) | 13.6 | 5.3-6.8 | 1982 |
| 10 | Restigouche (Kedgwick by 3 year virgin wild) | 7.4 | - | 1982 |
| 11 | Restigouche (Kedgwick wild) | 14.5 | - | 1981 |
| 12 | Rocky Brook (Wild) | 7.6 | - | 1982 |
| 13 | Tusket (Carlton wild) | 9.7 | 4.5-4.9<br>(5.0-5.8) | 1982 |
| 1, 2, 3, & 9 | Big Salmon River (Wild by hatchery) | 9.6 | - | 1982 |

* Estimates: personal communication, Dr. G. Farmer (1983), Farmer et al. (1980) and Watt et al. (1983).

Distribution

Trial No. I

| Tank | Unit No. 1 pH = 4.0*<br>Stock | Tank | Unit No. 2 pH = 4.0*<br>Stock |
|---|---|---|---|
| 11 | Big Salmon River 1<br>Big Salmon River 3<br>East River | 12 | Big Salmon River 1<br>Big Salmon River 2<br>East River |
| 21 | Big Salmon River 1<br>Big Salmon River 2<br>Medway | 22 | Big Salmon River 1<br>Big Salmon River 3<br>Margaree |
| 31 | Big Salmon River 1<br>Big Salmon River 2<br>Margaree | 32 | Big Salmon River 1<br>Big Salmon River 3<br>Liscombe |
| 41 | Big Salmon River 1<br>Big Salmon River 3<br>Liscombe | 42 | Big Salmon River 1<br>Big Salmon River 2<br>Medway |
| 51 | Big Salmon River 1<br>Big Salmon River 3<br>La Havre | 52 | Big Salmon River 1<br>Big Salmon River 2<br>LaHarve |

* pH control was erratic, the sensitivity was insufficient and slightly different between units.

Trial No. II

| Tank | Unit No. 1 pH = 3.80<br>Stock | Tank | Unit No. 2 pH = 3.88<br>Stock |
|---|---|---|---|
| 11 | Big Salmon 1<br>Big Salmon 9<br>Rocky Brook | 12 | Big Salmon 1<br>Big Salmon 3<br>Restigouche PYP |
| 21 | Big Salmon 1<br>Big Salmon 9<br>Tusket | 22 | Big Salmon 1<br>Big Salmon 9<br>Rocky Brook |
| 31 | Big Salmon 1<br>Big Salmon 3<br>Restigouche UPY[1] | 32 | Big Salmon 1<br>Big Salmon 3<br>Tusket |
| 41 | Big Salmon 1<br>Big Salmon 3<br>La Havre | 42 | Big Salmon 1<br>Big Salmon 3<br>Restigouche UPY |
| 51 | Big Salmon 1<br>Big Salmon 3<br>Restigouche PYP[2] | 52 | Big Salmon 1<br>Big Salmon 3<br>Restigouche PYP |

[1] Under yearling parr

[2] Post yearling parr

In order to correct for differences between both tanks and trials, Big Salmon River parr of common genotype were used. Twelve to fourteen parr from a single family (labeled one) were placed in every tank in all trials of the strain experiment. Twelve to fourteen parr from three other families were randomized, one family to a tank, in all trials. Thus, in addition to the test strain, each tank had two Big Salmon River families in it. One was used to correct for experimental errors, i.e. effects due to trial, unit, tank and length of fish, and others to provide an overall estimate of the adequacy of the correction.

The correction factors were calculated using the General Linear Models (GLM) procedure found in the Statistical Analysis System (SAS) Software package. The adequacy of the correction was tested using Randomized Block ANOVA on the test family (Huntsberger, 1969). This gave no significant difference for class (Trials, Units, etc.) or interaction.

This correction procedure was then used on all survival times for all stocks. A one-way ANOVA was used to test for survival time differences between stocks. Differences among stocks were determined using Duncan's multiple range test, a sub-routine of GLM. The concordance between family trials was checked using procedures of Snedecor and Cochran (1968).

### Acid Challenge

A random sample of offspring from each family was tested at acute hydrogen ion concentration, i.e. pH 4.2 or lower. The experiment was replicated three times in each year-class, and each replication was terminated, with one exception, when all of the fish in the tank were dead. The water calcium concentration was 3.4 ppm, and the conductivity was 34.2 UMNO/cm.

In the first test with the first generation, the alevins were placed in individual containers with the pH adjusted by hand twice a day. There were large swings in treatment pH. All other tests used fish identified as to family with a hot brand randomized to two of four 1.1 meter tanks. The pH was controlled automatically by metering sulfuric acid with a radiometer end point titration system (Table 2).

The same end point titration system was used to control pH in the stocks experiment. the pH was measured and sulfuric acid was metered into the header tank in each unit. To minimize nitrogen buildup, no feeding was done during the trials. Nitrogen levels did not exceed normal lake levels. The calcium ion concentration was 3.8 ppm, and the conductivity was 26 UMNO/cm.

## RESULTS AND DISCUSSION

### Family Trials

Fish tested in April, 1981, prior to first feeding, ranged in mean survival time from 123.1 hours to 140.0 hours. The ranking remained essentially the same when they were tested at 9 months and 10 months post-hatch with concordance significant at the 5% level between trials. The only major exception was family 156 that ranked 6th for trial one and first in trials two and three (Table 3). This difference can probably be explained by the

TABLE 2 THE EXPERIMENTAL CONDITION USED FOR THE FAMILIES TRIALS

| Generation | Trial | Number tested per family | Age | pH |
|---|---|---|---|---|
| 1 | 1 | 300 | Alevin | 4.3 |
| | 2 | 150 | 9 month post hatch | 3.7 |
| | 3 | 150 | 10 months post hatch | 3.2 |
| 2 | 1 | 300 | 3 months post hatch | 3.9 |
| | 2 | 150 | 5 months post hatch | 4.0 |
| | 3 | 150 | 6 months post hatch | 3.7 |

TABLE 3 MEAN SURVIVAL TIMES AND RANKING OF 1980 YEAR CLASS FAMILY OFFSPRING. THE FIRST TRIAL WAS RUN ONE MONTH POST HATCH, THE OTHER TWO, 8 AND 9 MONTHS POST HATCH, RESPECTIVELY. THESE ARE THE UNCORRECTED SURVIVAL TIMES. THE CONCORDANCE BETWEEN RANK WAS ALMOST COMPLETE BEING SIGNIFICANT AT THE 5% LEVEL BETWEEN APRIL AND BOTH DEC. AND JAN. IT WAS SIGNIFICANT AT THE 1% LEVEL BETWEEN DEC. AND JAN.

| Identification | April pH 4.3 at 13.5° C Rank (Time) | Dec. pH 3.7 at 8.5° C Rank (Time) | Jan. pH 3.2 at 4.0° C Rank (Time) |
|---|---|---|---|
| 143 | 1 (140.0) | 2 (143.1) | 2 (37.4) |
| 151 | 2 (134.9) | 3 (140.0) | 3 (37.1) |
| 152 | 3 (132.2) | 5 (116.4) | 4 (35.2) |
| 148 | 4 (130.2) | 6 (76.2) | 6 (33.8) |
| 147 | 5 (127.1) | 4 (129.1) | 5 (34.7) |
| 156 | 6 (123.1) | 1 (165.2) | 1 (38.3) |

fact that family 156 had developed slightly faster than the other families and was partially starved at the time of the trial. When it was removed, the significance level increased to the 1% level.

This consistency in rank is particularly intriguing because the test conditions varied markedly between between trials (see Tables 2 & 3 for details). Because the relative response of the families -- the family rank -- is consistent; therefore, independent of the environment, it must be genetically controlled and likely by one set of alleles. If a different set of alleles operated at lower pH than at higher pH, then rank should change, as there would be no reason to expect the high resistance controlling elements to be present in the same family in the same ratio for different physiological mechanisms, i.e. different responses to different pH. This implies that the physiological response, possibly a generalized stress response (Wood and McDonald, 1982) leads to death through a set of steps, each driving the system further out of equilibrium, i.e. positive feed back.

Genetic control of resistance to low pH is further emphasized by the comparable ranking of parent family and offspring family. The concordance was significant at the 1% level (Table 4) even though the trials were run on fish of different ages and under different experimental conditions than the previous years' trials (Table 4). This is particularly intriguing, as the genes for resistance come through the males only, i.e. males were randomized to females in making the matings.

TABLE 4 MEAN SURVIVAL TIME AND RANK FOR 1980 YEAR CLASS TRIAL NO. 1 THE PARENT GENERATION, AND THE 1981 YEAR CLASS, THE OFFSPRING GENERATION. THE CONCORDANCE BETWEEN RANKS WAS ALMOST COMPLETE BEING SIGNIFICANT AT THE 1% LEVEL

| Line | Parent Family | | Offspring | | |
|---|---|---|---|---|---|
| | Rank | Mean standardized Survival Time | Rank | Number used as sires | Mean standardized Survival Time (hr) |
| Control (wild) | | -- | -- | 13 | 132.3 |
| Mean | | 131.3 | -- | -- | 131.2 |
| 143 | (1) | 140.0 | (2) | 10 | 136.9 |
| 151 | (2) | 134.9 | (1) | 2 | 144.8 |
| 152 | (3) | 132.2 | (6) | 1 | 112.9 |
| 143 | (4) | 130.2 | (4) | 4 | 126.5 |
| 147 | (5) | 127.1 | (5) | 5 | 120.7 |
| 156 | (6) | 123.1 | (3) | 4 | 129.1 |

Genetic control of mortality seemed to be reflected not only in the absolute length of time the longest-lasting individual survived, but also in the proportion dying after different times (Figure 1). This family specific distribution was relatively constant from trial to trial. As the distributions were more complex than could be accounted for by a one-gene, two-allele model, this implies multiple gene control. Secondly, as the distributions vary markedly, no one tansformation was appropriate; thus, a normal distribution was the best overall approximation.

### Strains Trial

The mean was a better estimator of the central tendency than the median, the $LT_{50}$. The mean does respond to deviations from normality. For example, stock 5 (Table 5) has a corrected mean of 39 hours and a corrected median value of 57.6 hours. Stock 8, Trial 2, Unit 1 (Figure 2) has an uncorrected median of 24 hours and an uncorrected mean of 28.6 hours. The number dying, either early or late in the trial, weight the mean, making it a better estimate of the relative tendency to survive. In addition, genetic analysis can be done using means and variance, but not the median.

The Big Salmon River fish formed an experimental block in the strain trials. Their rank, based on means from highest to lowest, 1, 2, 3, 9 (Table 5), was consistent with other trials (unpublished data).

The La Have stock was the outstanding performer, having a survival time significantly better than any other stock. The East River and the Liscombe stock were the worst. The other stocks formed three statistically significant additional groups (Table 5).

Applying acute treatments of different severity (pH 4.0 and 3.88) did not seem to affect the relative ranking of stocks any more than it did families. The more severe treatments did, however, change the distribution of individuals within the stocks trial, as it did the relative rate at which some of the lower ranking family mortalities occurred in the families trial. Thus, the clumping which formed two modes in the La Have stock (Figure 2) probably represented the separation of individuals into groups containing the least resistant individuals and the most resistant individuals. If, as indicated above, the control is genetic, then a short pH shock should be sufficient to eliminate low resistant individuals early in the life history. This could be of significance if acute and chronic responses correspond and consideration is given to restocking marginal systems.

### Interpretation

To interpret these data, a number of assumptions must be accepted. First, the analytic technique was adequate. As indicated above, it was for the Big Samon River fish and should appear to be for all sets of data because only 3 of 60 corrections lead to a shift in rank of means within a Trial, Unit, Tank group.

A second assumption was that the genetic responses noted in the Big Salmon River fish (the base line fish) also held for the other strains. For example, the length of the fish was significant only at the 10% level in the base line family, thus of relatively little importance. This point was of particular concern because Daye and Garside (1977) reported that older, larger fish showed more resistence than younger, smaller fish. Because the fish in this study varied in size and age even though within the Big Salmon

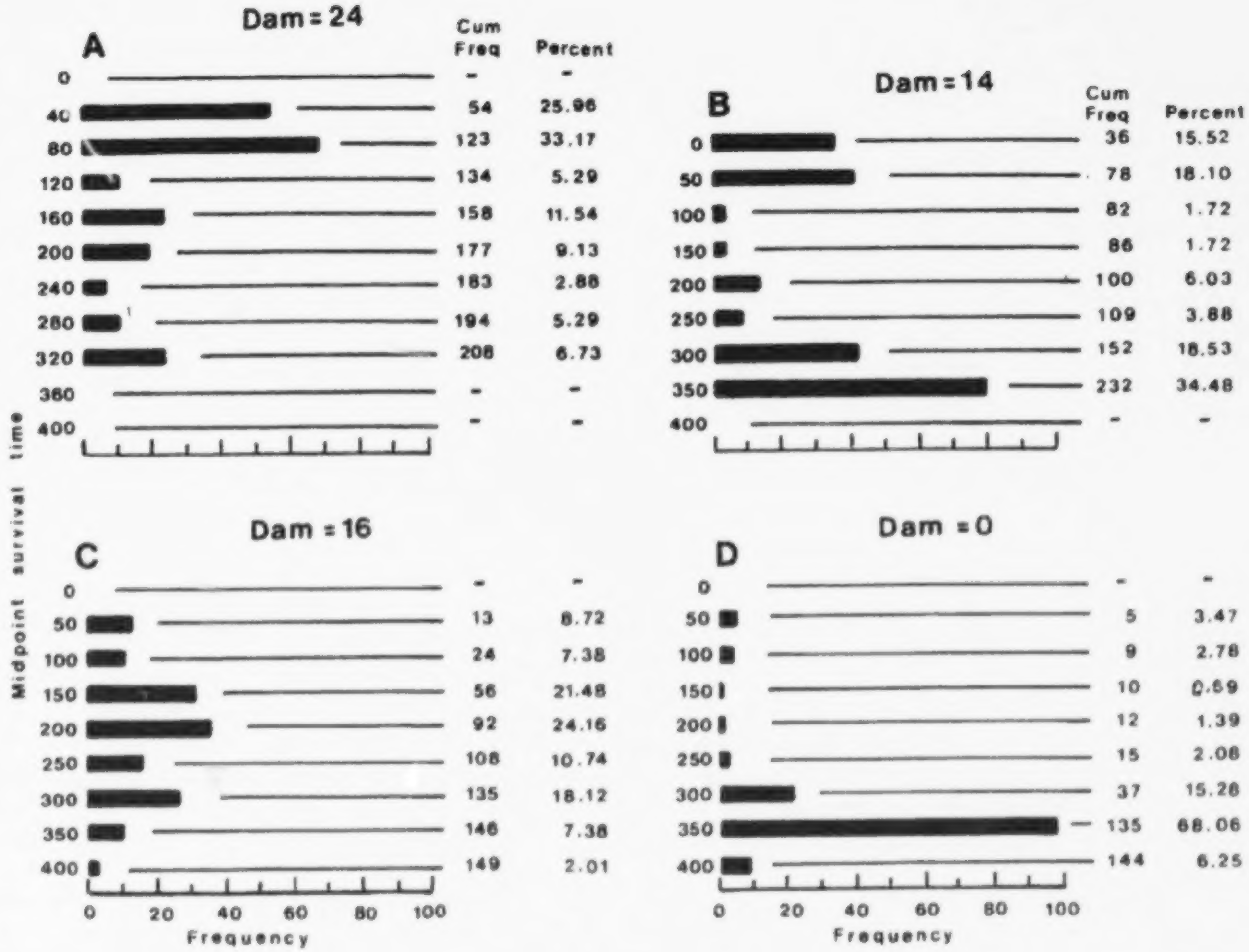

FIGURE 1 SURVIVAL TIME FREQUENCY DISTRIBUTION OF FOUR BIG SALMON RIVER OFFSPRING FAMILIES. THESE GRAPHS ILLUSTRATE THE RANGE OF VARIABILITY WITHIN FAMILIES

TABLE 5 THE MEAN SURVIVAL TIME CORRECTED FOR TRIAL, UNIT, TANK AND LENGTH OF THE INDIVIDUAL FISH. THE RANKING USED DUNCAN'S MULTIPLE RANGE TEST WITH THE ANALYSIS OF VARIANCE

| Identification Number | Stock | Number Tested | Mean Time Hours | Grouping* | $LT_{50}$** |
|---|---|---|---|---|---|
| 8 | La Havre (Hatchery) | 188 | 84.9 | A | 85.7 |
| 6 | Medway (wild) | 112 | 75.8 | B | 77.4 |
| 11 | Restigouche (PYP) (Kedgwick wild) | 65 | 71.7 | B | 71.6 |
| 13 | Tusket (Carleton wild) | 115 | 64.4 | C | 61.5 |
| 10 | Restigouche (UYP) (Kedgwick by 3 year virgins) | 99 | 55.9 | D | 55.7 |
| 1 | Big Salmon River | 258 | 55.2 | D | 58.9 |
| 2 | Big Salmon River | 66 | 54.3 | D | 61.4 |
| 3 | Big Salmon River | 123 | 54.2 | D | 61.9 |
| 9 | Big Salmon River | 73 | 54.1 | D | 54.1 |
| 12 | Rocky Brook (Wild) | 96 | 50.5 | D | 51.3 |
| 5 | Margaree (Wild) | 18 | 39.0 | E | 57.6 |
| 7 | East River (Kelt Hatchery) | 45 | 23.5 | F | 46.8 - 49.1 |
| 4 | Liscombe (Hatchery) | 51 | 21.0 | F | 41.8 - 44.6 |

* Different letters indicate significant differences at the 5% level.

** Time to 50% mortality.

River set, no significance for length effects could be found, a length correction was made using GLM.

An additional assumption necessary was the extension of the single set of genes controlling the response to acute pH argument to chronic levels pH. Both Swartz et al. (1978) and Rahel and Magnuson (1983) supported this by reporting correspondence between

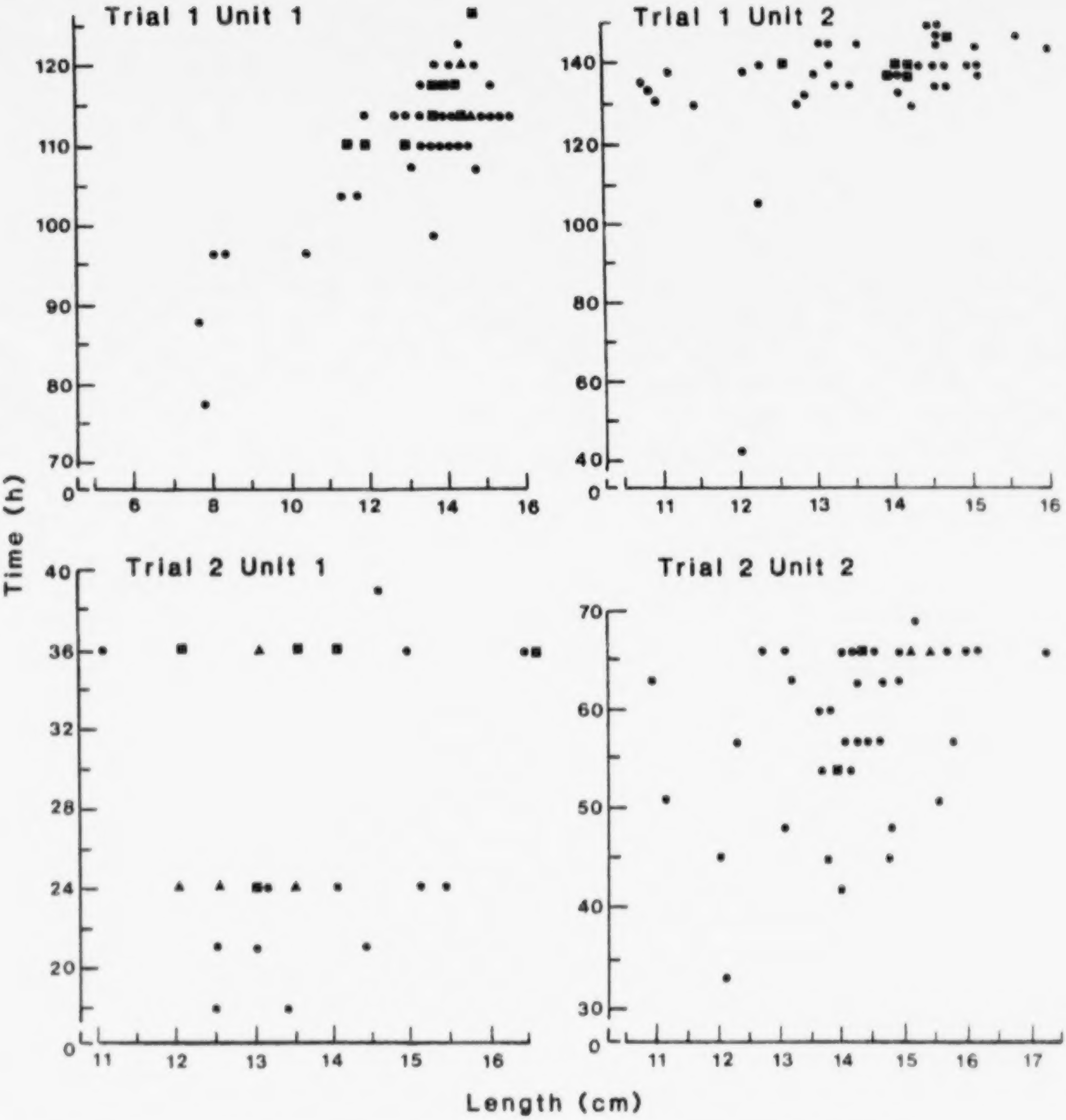

FIGURE 2 THE LENGTH SURVIVAL TIME DISTRIBUTION FOR STOCK 8 (LA HAVRE). THE MOST SEVERE CONDITIONS GIVE SEPARATION INTO TWO GROUPS. NOTE ALSO THAT WHILE LENGTH DOES AFFECT SURVIVAL IT IS ONLY A MODERATE INFLUENCE. THE pH'S USED WERE, IN TRIAL I, UNIT 1, 4.0; UNIT 2, 4.0 AND IN TRIAL II, UNIT 1, 3.8 AND UNIT 2, 3.88. THE . SIGNIFIES 1 OBSERVATION, THE , 2 OBSERVATIONS AND THE , 3 OBSERVATIONS

chronic and acute responses in Brook trout and Yellow perch, respectively. It seemed reasonable to argue that declining pH in native and/or hatchery systems (Thompson et al. 1980) and Watt et al. 1983 and Gough, 1983 Personal Communication) caused changes in stock genotype such that the La Have, Medway and Tusket stocks were the most resistant of those tested. Thus, low pH in nature or hatchery would have applied a selection pressure favouring more resistant offspring and provide evidence, however circular, for a correspondence between response to chronic and acute levels of pH. On the other hand, the relatively poor performance of the Margaree, East River and Liscombe fish may be unreliable because they had relatively high mortalities attributable to transportation stress.

Caution must also be exercised in attempting to relate results reported here to results in wild populations, as the fish tested were all hatchery stocks. The hatchery environment may have inadvertantly applied selection pressure, removing some genotypes. However, the La Have stock would have to be the clear choice to restock a river system with marginal pH based on these data.

## SUMMARY

There is genetic control over the resistance to low pH. The genetic mechanism for this resistance is likely the same at acute and chronic levels of pH with the responses under the control of one set of genes. The different levels of resistance and the mortality rates (measured at acute pH) can probably be used to predict performance at chronic pH.

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# EFFECT OF LOW PH ON GONADAL DEVELOPMENT OF BROOK TROUT (SALVELINUS FONTINALIS): RESULTS FROM FIELD STUDIES DONE ON ONTARIO LAKES IN THE SAULT STE MARIE AND BLIND RIVER AREAS.

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ROY, R.J.J. and W.H. TAM. 1985. Effect of low pH on gonadal development of brook trout (*Salvelinus fontinalis*) = results from field studies done on Ontario lakes in the Sault Ste Marie and Blind River areas. Can. Tech. Rep. Fish. Aquat. Sci. 1368: p. 149-150.

Brook trout were netted during the summers of 1981-1983 from lakes whose pHs ranged from 5.5 to 7.5. Gonadal steroid biochemistry and plasma estrogen levels were measured in the female, and gonadal development was determined by histology in both sexes. Results from the Sault Ste Marie area lakes surveyed in 1981 suggest that oocyte and testicular development are correlated to lake pH. As lake pH decreases, so does the proportion of yolky eggs in the ovary and proportion of maturing spermatocytes and spermatozoa in the testis. A similar pattern occurred in fish taken from one acidic and one neutral lake in the Blind River area, but differences in gonadal development in these trout are not statistically significant. However, plasma estrogen levels during the spawning period were significantly higher in trout from the acidic lake, probably due to the presence of unovulated eggs. Results from vitellogenin determinations in plasma and from the 1983 field study will be presented and discussed.

ROY, R.J.J. and W.H. TAM. 1985. Effect of low pH on gonadal development of brook trout (*Salvelinus fontinalis*) = results from field studies done on Ontario lakes in the Sault Ste Marie and Blind River areas. Can. Tech. Rep. Fish. Aquat. Sci. 1368: p. 149-150.

Des ombles de fontaine ont été rassemblées au filet au cours des étés 1981, 1982 et 1983, en provenance de lacs dont le pH variait entre 5,5 et 7,5. La biochimie des stéroides gonadiques et les niveaux d'oestrogène plasmatique ont été mesurés chez la femelle et, dans les deux sexes, le développement gonadique a été déterminé par examen histologique. Les résultats des lacs de la région de Sault-Sainte-Marie recueillis en 1981 semblent indiquer que le développement des testicules et de l'oocyte sont en corrélation avec le pH du lac. Lorsque le pH du lac diminue, on observe également une diminution de la proportion des oeufs chargés de vitellus dans l'ovaire, une diminution de la proportion des spermatocytes en maturation ainsi que des spermatozoides dans le testicule. Les mêmes observations se présentent chez les poissons pris d'un lac acide ou d'un lac neutre dans la région de Blind River, Mais les différences dans le développement gonadique de ces ombles ne sont pas statistiquement significatives. Cependant les niveaux d'oestrogène plasmatique pendant la période de frai ont été significativement plus élevés chez les ombles provenant du lac acide, probablement en raison de la présence d'oeufs non ovulés.

Nous présenterons et examinerons les résultats provenant des déterminations de vitellogénine dans le plasma et à partir de l'étude sur le terrain de 1983.

# EFFECTS OF ACIDIC pH ON GROWTH AND BEHAVIOR OF BLACKNOSE DACE, SLIMY SCULPINS AND JUVENILE ATLANTIC SALMON IN A SIMULATED STREAM ENVIRONMENT

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TOWNSEND, D. and D. HOOD. 1985. Effects of acidic pH on the growth and behavior of blacknose dace, slimy sculpins and juvenile Atlantic salmon in a simulated stream environment. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 151-152.

Preliminary results will be presented from an on-going investigation of the effects of sublethal levels of acidic pH on growth and intra- and interspecific behavior of juvenile Atlantic salmon and two sympatric stream fish species, blacknose dace and slimy sculpins. Tests were conducted with treatment pH levels of 5.0, 5.5, 6.0 and 6.5 in four large laboratory stream tanks set up to simulate salmon nursery habitat. Water taken from a salmon stream was used in the experiments. For each of the replicates of the experiment, a "resident" population of wild fish of all three species was established then, after seven days, hatchery-reared salmon fry were "stocked" in each tank. Population densities used were equivalent to those found in the wild. To date, at all pH levels, intra- and interspecific interactions have been similar. Small salmon parr (0+ and 1+ fish) actively defend areas of the tank. They consistently hold position within these territories at specific locations (stations) and they attack other small parr, blacknose dace, sculpins and, occasionally, the larger parr (1+ and 2+ fish) that linger near them. The larger parr, although maintaining stations, less consistently defend the area around them. Blacknose dace and sculpins consistently flee when attacked and sometimes are pursued for short distances. No social interactions between dace and sculpins have been observed although the two species were sometimes observed in close proximity to one another. Little predation of any species appears to have occurred; almost all fish have been accounted for in our experiments. Although few deaths of other species have occurred at any pH level, at the pH 5.0, the incidence of deaths of sculpins increased markedly over that occurring at other pH levels.

Feeding activity was scored only for the juvenile salmon. No differences in feeding activity between treatment levels were observed for the large parr and hatchery-reared small parr, however, for the small wild parr, feeding frequency differed significantly at the different pH levels. No statistically significant relationship between weight gain and pH has been found. The frequency with which wild parr moved from one station to another within their territory was similar at all pH levels, however, hatchery-reared small parr were significantly more active in changing stations at the highest pH (6.5) than at all other pH levels. Similar frequencies of agonistic behaviours (occurring primarily during territorial interactions) were shown by wild and hatchery parr at all pH levels.

A test of sublethal copper concentrations, as a factor affecting juvenile Atlantic salmon behavior and growth at different pH levels in stream water, is currently being conducted. The results will be used to assess potential confounding effects of sublethal concentrations of copper encountered in some of the experimental replicates.

TOWNSEND, D. and D. HOOD. 1985. Effects of acidic pH on the growth and behavior of blacknose dace, slimy sculpins and juvenile Atlantic salmon in a simulated stream environment. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 151-152.

Nous présenterons les résultats préliminaires obtenus à partir d'une étude en cours sur les effets de niveaux sublétaux de pH acide sur la croissance et le comportement intra et interspécifique du saumon de l'Atlantique juvénile et de deux espèces fluviales sympatriques, le naseux noir et le chabot visqueux. Les essais ont été conduits avec des pH de 5,0, 5,5, 6,0 et 6,5, dans quatre réservoirs de laboratoire à courant simulant l'habitat d'élevage du saumon. Pour cette expérience, on a utilisé de l'eau provenant d'une rivière à saumon. Pour chacune des répétitions de l'expérience, une population résidente de poissons sauvages des trois espèces a été établie; puis, après sept jours, chaque réservoir a été peuplé de jeunes saumons d'alevinage. Les densités de population utilisées étaient équivalentes à celles qu'on trouve dans la nature. Jusqu'à ce jour, à tous les niveaux de pH, les interactions intra et interspécifiques sont similaires. Les tacons (0+ et 1+ an) défendent activement les zones du réservoir. De façon persistante, ils tiennent leur position à l'intérieur de ces territoires à des endroits déterminées (postes) et ils attaquent les autres tacons, les naseux noirs, les chabots, et, occasionnellement, les grands tacon (1+ et 2+ an) qui se tiennent autour d'eux. Le tacon plus développé défend ses postes, mais défend moins activement les zones avoisinantes.

De façon constante, le naseux noir et le chabot s'enfuient lorsqu'ils sont attaqués, et ils sont quelquefois poursuivis sur de courtes distances. On n'a pas observé d'interaction sociale entre naseux noir et chabot, mais les deux espèces ont été quelquefois observées à proximité l'une de l'autre. Il n'est apparu que peu de prédation des espèces; presque tous les poissons étaient présents dans nos expériences. Bien qu'il y ait peu de décès chez les autres espèces à quelque niveau de pH que ce soit, au pH 5,0 la mortalité chez les chabots a augmenté de façon marquée par rapport à celle des autres niveaux de pH.

L'activité alimentaire a été notée seulement pour le jeune saumon. On n'a observé aucune différence d'activité alimentaire entre les niveaux de traitement, pour le tacon déjà développé et pour le petit tacon d'alevinage; cependant, pour le petit tacon sauvage, la fréquence d'alimentation a varié considérablement aux différents niveaux de pH. Aucun relation statistiquement significative n'a été trouvée entre le gain de poids et le pH. La fréquence à laquelle le tacon sauvage se déplace d'une station à l'autre à l'intérieur de son territoire a été similaire à tous les niveaux de pH; cependant, le petit tacon d'alevinage a été significativement plus actif dans ses déplacements au pH le plus élevé (6,5) qu'aux autres niveaux de pH. Des fréquences similaires de comportement agonistique (se produisant principalement pendant les interactions territoriales) se sont présentées chez le tacon sauvage et le tacon d'alevinage à tous les niveaux de pH.

Nous sommes actuellement en train de faire un test de concentrations sublétales de cuivre, comme facteur influençant le comportement et la croissance du saumon de l'Atlantique juvénile à différents niveaux de pH en eau de rivière. Les résultats seront utilisés pour évaluer les effets potentiels déconcertants des concentrations sublétales de cuivre observées au cours certaines des expériences renouvelées.

# LABORATORY STUDIES ON ZYGNEMATACEAN ALGEA: THE GROWTH OF MOUGEOTIA SPP. IN INORGANIC MEDIUM AT VARIABLE PH.

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TURNER, P.A.E. and P.M. STOKES. 1985. Laboratory studies on Zygnematacean algae: the growth of Mougeotia spp. in inorganic medium at variable pH. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 153-154.

It has been noted in the literature that lake acidification may sometimes be associated with an increase in the extent of communities of attached filamentous algae. Several authors have suggested decreased heterotrophic activity, rather than increased algal productivity, as being the mechanism involved. In addition, many acidified lakes show increased levels of aluminum which may further influence algal growth. The objective of this work is to compare the growth of pure culture of Mougeotia spp. from acidic lakes in medium at different pH values and at different levels of aluminum.

We have successfully isolated pure cultures of Mougeotia spp. from Chub Lake (Dorset, Ont.; pH 5.3) and Lake Ruth Roy (Killarney, Ont.; pH 4.5). To date the growth response of one clone from each lake has been measured in inorganic medium at pH values of 4.5, 5.6, and 6.7.

Growth was measured by dry weight and total filament length per flask. Filament length was measured in an attempt to increase the sensitivity of growth measurements as dry weight becomes detectable only towards the end of the growth period. The Chub Lake culture showed a measurable increase in dry weight after 8 days in medium at pH values of 5.6 and 6.7 but not at pH 4.6. Variation was reduced up to and including day 12 and increased thereafter. Dry weights of algae grown in medium at pH 5.6 were always slightly greater than at 6.7 but the difference was not significant. Algae at pH 4.6 showed little detectable increase in dry weight over 16 days.

Dry weights of the Lake Ruth Roy clone were not detectable until day 9 but variation was considerable, while total filament length per flask increased considerably by day 8 in medium at pH 4.6 and 5.6 but not 6.7. Total filament length was slightly greater in medium pH 5.6 compared to 4.6 while it was completely reduced at pH 6.7 for the 12 days of the experiment.

These results indicate that the clone of Mougeotia spp. from Chub Lake may be less acid tolerant to low pH conditions than the clone from more acidic Lake Ruth Roy. It appears that neither clone shows a preference for the low pH which adds support to the suggestions of decreased heterotrophic removal of filamentous algae in low pH.

TURNER, P.A.E. and P.M. STOKES. 1985. Laboratory studies on Zygnematacean algae: the growth of Mougeotia spp. in inorganic medium at variable pH. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 153-154.

On peut constater dans la documentation que l'acidification des lacs peut quelquefois être associée avec un accroissement des communautés d'algues filamenteuses attachées. Cerains auteurs parlent de décroissance de l'activité hétérotrophique plutôt que d'accroissement de productivité des algues. De plus, de nombreux lacs acides présentent un accroissement du niveau d'aluminium qui peut à son tour influencer la croissance des algues. L'objectif de nos travaux est de comparer la croissance de cultures pures de Mougeotia provenant de lacs acides dans des milieux à différents pH et à différents niveaux d'aluminium.

Nous avons isolé avec succès des cultures pures de Mougeotia à partir du lac Chub (Dorset (Ontario); pH 5,3) et du lac Ruth Roy (Killarney (Ontario); pH 4,5). Jusqu'à présent, la réaction de croissance d'un clone de chaque lac a été mesurée dans un milieu inorganique à des pH de 4,5, 5,6, et 6,7.

La croissance a été mesurée par le poids sec et la longueur totale des filaments par flacon. La longueur des filaments a servi à augmenter la précision des mesures de croissance, car le poids sec est décelable seulement vers la fin de la période de croissance. La culture provenant de lac Chub a présenté un accroissement mesurable en poids sec après 8 jours dans le milieu à des pH de 5,6 et 6,7, mais pas au pH 4,6. Cette variation s'est réduite jusqu'au 12$^{e}$ jour inclusivement, puis s'est accrue par la suite. Le poids sec des algues poussant dans le milieu à pH 5,6 a toujours été légèrement plus grand qu'à pH 6,7, mais cette différence n'était pas significative. Les algues au pH 4,6 ont montré une augmentation peu perceptible du poids sec après 16 jours.

Les poids secs du clone du lac Ruth Roy n'ont pas été décelables avant le 9$^{e}$ jour, mais la variation a été considérable, tandis que la longueur totale des filaments par flacon augmentait de façon importante au 8$^{e}$ jour dans les milieux à pH 4,6 et 5,6 mais pas à pH 6,7. La longueur totale des filaments était légèrement plus importante dans le milieu à pH 5,6 qu'à pH 4,6 tandis qu'elle était complètement réduite à pH 6,7 pendant les 12 jours de l'expérience.

Ces résultats indiquent que le clone de Mougeotia provenant du lac Chub tolère probablement moins l'acidité dans des conditions de pH faibles, que le clone provenant du lac acide Ruth Roy. Il apparaît qu'aucun des clones ne présente une préférence pour le pH faible, ce qui vient confirmer les hypothèses de diminution de l'activité hétérotrophique des algues filamenteuses à pH faible.

# ENERGY METABOLISM DURING SMOLTIFICATION OF *SALMO SALAR* UPON EXPOSURE TO LOW pH UNDER LABORATORY AND HATCHERY CONDITIONS

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WAIWOOD, B.A., K. HAYA, and L. VAN EECKHAUTE. 1985. Energy metabolism during smoltification of *salmo salar* upon exposure to low pH under laboratory and hatchery conditions. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 155-156.

Acid precipitation has resulted in the loss of salmonid populations in many areas of N.W. Europe and N.E. America. Saunders et al. (1982) have shown that low pH interferes with the smoltification of *Salmo salar*, one of the main effects being the inhibition of adenosine triphosphatase activity in gill filament tissue. This study was designed to determine the effects of low pH on intermediary energy metabolism during smoltification under laboratory and hatchery conditions.

The laboratory study was part of a larger experiment conducted in St. Andrews using acid-treated water at pH 4.5 and the normal water supply from Chamcook Lake at pH 6.5. The hatchery study was held at the Mersey Fish Hatchery, also part of a larger study, and included three exposure conditions, normal hatchery water at pH 4.9, acid-treated water at pH 4.5 and lime-treated water at pH 6.0. The laboratory study involved the determination of adenylates, glucose, glycogen, creatine phosphate and inorganic phosphate in liver and muscle tissue while in the field study only liver tissue was sampled.

Analysis of length, weight and liver weight records for fish sampled during the 83-day experiment showed significant decreases in condition factor, liver somatic index and lack of growth in acid-exposed fish. Statistical analysis of the biochemical data for the laboratory study shows significant differences in adenosine triphosphate, adenosine diphosphate, adenylate energy charge, creatine phosphate, glucose and glycogen in muscle tissue of acid-exposed salmon as compared to the control fish.

In liver tissue adenosine triphosphate, total adenylates, adenylate energy charge and glucose were consistently higher in acid-exposed salmon, indicating a decrease in anabolic processes which contributes to the detrimental effects of exposure to low pH. The values of the biochemical parameters from the Mersey Fish Hatchery study will be compared with the laboratory results.

WAIWOOD, B.A., K. HAYA, and L. VAN EECKHAUTE. 1985. Energy metabolism during smoltification of *salmo salar* upon exposure to low pH under laboratory and hatchery conditions. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 155-156.

Les pluies acides ont entraîné une baisse de la population de saumons dans de nombreuses régions de l'Europe du nord-ouest et de l'Amérique du nord-est. Saunders et

coll. (1982) ont montré que des pH faibles perturbent la smoltification de Salmo salar, un de leurs effets principaux étant l'inhibition de l'activité de l'adénosine triphosphatase dans les tissus filamenteux des branchies. Cette étude a pour objectif de déterminer les effets des pH faibles sur le métabolisme énergétique intermédiaire pendant la smoltification en laboratoire et en bassin d'alevinage.

L'étude en laboratoire a fait partie d'une expérience de grande envergure conduite à St. Andrews et utilisant de l'eau traitée à l'acide, à un pH de 4,5, et de l'eau normale provenant du lac Chamcook, à un pH de 6,5. L'étude en bassin d'alevinage a eu lieu au centre piscicole Mersey Fish, dans le cadre de travaux plus étendus, et comportait trois conditions d'exposition: de l'eau d'alevinage normale à un pH de 4,9; de l'eau acide, à un pH de 4,5; et de l'eau traitée à la chaux, à un pH de 6,0. L'étude en laboratoire comprenait la détermination des niveaux d'adénylates, de glucose, de glycogène, de phosphate de créatine et de phosphate inorganique dans les tissus du foie et des muscles, tandis que dans l'étude sur le terrain, seuls les tissus hépatiques étaient recueillis.

L'analyse de la taille, du poids des poissons ainsi que du poids de leur foie pendant les 83 jours de l'expérience a montré une diminution significative du facteur de condition, de l'index somatique du foie et de l'absence de croissance chez les poissons exposés à l'acide. L'analyse statistique des données biochimiques fournies par l'étude de laboratoire a montré des différences significatives dans la charge d'énergie de l'adénosine triphosphate, de l'adénosine diphosphate et de l'adénylate, ainsi que dans les niveaux de phosphate de créatine, de glucose et de glycogène dans les tissus musculaires du saumon exposé à l'acide, par rapport aux poissons témoins.

Dans le tissu hépatique, la charge d'énergie des triphosphates, des adénylates totaux, et de l'adénylate, ainsi que le niveau de glucose, ont été constamment plus élevés chez le saumon exposé à l'acide, ce qui indique une baisse des processus anaboliques contribuant aux effets défavorables de l'exposition à de faibles pH. La valeur des paramètres biochimiques provenant de l'étude de pisciculture Mersey Fish sera comparée aux résultats de laboratoire.

# BIOCHEMICAL TOXICOLOGY

Jerry Payne, Chairman

# ACCLIMATION OF RAINBOW TROUT TO ZINC -- KINETICS AND MECHANISM OF TOLERANCE INDUCTION

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BRADLEY, R.W., C. DUQUESNAY, and J.B. SPRAGUE. 1985. Acclimation of rainbow trout to zinc - kinetics and mechanism of tolerance induction. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 159-160.

Rainbow trout exposed to a sublethal level of zinc equal to 1/3 to 1/2 their LC50 showed a 2.5-fold increase in zinc tolerance during subsequent lethal tests. This enhanced tolerance was induced within 5 days, was maintained through 20 days of the sublethal exposure, and was lost within 7 days when the fish were returned to zinc-free water.

Levels of zinc in gill tissue of the acclimated fish were unchanged after 5 days, but did show small and statistically-significant increases at 12 and 20 days of exposure. No significant changes in zinc levels were observed in liver tissue of acclimated fish.

In a separate, parallel experiment, levels of heat-stable, sulfhydrylrich protein (HSP) were measured in liver and gill tissue of fish exposed to a zinc concentration of approximately 1/3 their LC$_{50}$. HSP levels increased by a factor of approximately 1.8 after 5 days of zinc exposure. This increase was also maintained during 20 days of zinc exposure and lost 5 days after the fish were placed in zinc-free water.

These results suggest that while HSP may play a role in the induction of enhanced tolerance of fish to zinc, the protein is not simply binding the incoming metal, since zinc accumulation in tissues of acclimated fish did not correlate with either HSP levels or tolerance. It is suggested that if HSP is involved in the induction of increased tolerance, its role is one of increasing the ability of a given tissue to regulate zinc levels, thus preventing the accumulation of excessive amounts of zinc. This hypothesis is supported by the observation that acclimated fish accumulated zinc more slowly in gill tissue than did control fish.

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La truite arc-en-ciel exposée à un niveau sublétal de zinc égal au tiers ou à la moitié de la CL50 a montré un accroissement de 2,5 fois sa tolérance au zinc pendant les tests létaux qui ont suivi. Cette augmentation de la tolérance a été induite en 5 jours, s'est maintenue pendant 20 jours d'exposition sublétale, et a disparu dans les 7 jours qui ont suivi le retour des poissons à une eau dépourvue de zinc.

Le niveau du zinc dans le tissu des branchies du poisson acclimaté ne s'est pas modifié après 5 jours mais, après 12 à 20 jours d'exposition, a montré des augmentations faibles, mais statistiquement significatives. Dans le tissu hépatique des poissons acclimatés, aucune modification significative du niveau du zinc n'a été observée.

Dans une expérience parallèle distincte, on a mesuré les niveaux de protéine riche en sulfhydryle, thermostable, dans les tissus du foie et des branchies du poisson exposé à une concentration de zinc d'environ le tiers de la CL50. Les niveaux de proteine riche en sulfhydryle ont augmenté d'un coefficient d'environ 1,8 après 5 jours d'exposition au zinc. Cet accroissement s'est également maintenu pendant les 20 jours d'exposition au zinc et a disparu après 5 jours dans l'eau sans zinc.

Ces résultats semblent indiquer que la proteine riche en sulfhydryle peut jouer un rôle dans l'induction d'un accroissement de tolérance du poisson au zinc, mais que cette protéine ne fixe pas seulement le métal introduit, puisque l'accumulation de zinc dans le tissu du poisson acclimaté n'est en corrélation ni avec les niveaux de protéine riche en sulfhydryle, ni avec la tolérance. Nous pouvons supposer que si la protéine riche en sulfhydryle joue un rôle dans l'induction d'une tolérance accrue, il consiste principalement à augmenter la capacité d'un tissu donné de régler les niveaux de zinc et, ainsi, de prévenir l'accumulation de quantités excessives de zinc. Cette hypothèse est corroborés par l'observation que le poisson acclimaté accumule le zinc dans ses branchies plus lentement que ne le fait le poisson témoin.

# THE USE OF PLASMA LEUCINE AMINO NAPHTHYLAMIDASE (PLAN) AS AN INDICATOR OF TOXICANT STRESS IN RAINBOW TROUT

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DIXON, D.G., P.V. HODSON, and K.L.E. KAISER. 1985. The use of plasma leucine amino naphthylamidase (PLAN) as an indicator of toxicant stress in rainbow trout. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 161-162.

Studies were undertaken to evaluate the potential application of changes in the plasma level of the proteolytic enzyme leucine amino naphthylamidase to assessing acute sublethal toxicant impact in rainbow trout. Blood samples were taken from groups of 80-100 g fish following intraperitoneal and/or waterborne exposure to para-methylphenol (PMP) or carbon tetrachloride ($CCl_4$).

The duration of post injection holding was found to have a significant impact on PLAN activity in control and PMP-dosed rainbow trout with the largest increase after 96 h. Statistically significant relationships between dose and PLAN activity were obtained for both PMP and $CCl_4$ with intraperitoneally injected fish. PMP dosed fish showed PLAN levels elevated by 27 to 63% relative to controls 96 h after injection. Fish dosed with $CCl_4$ showed PLAN levels 38 to 135% higher than controls 48 h after injection. A waterborne concentration of 0.028 mM PMP (0.41 96 h LC50) resulted in statistically significant increases in the PLAN activity shown by rainbow trout after 48, 96 and 192 h of exposure. PLAN activity increased by 38 to 87% relative to controls. In all cases, changes in plasma protein level and liver: body-weight ratio tended to mirror changes in PLAN activity, although no significant histopathological lesions in liver tissue were evident.

The temperature and duration of plasma storage were found to have a significant effect on the PLAN activity shown by control and PMP-dosed rainbow trout. While diet was found to be a significant modifier of PLAN activity, sex had no effect. Potential applications of PLAN activity will be discussed.

DIXON, D.G., P.V. HODSON, and K.L.E. KAISER. 1985. The use of plasma leucine amino naphthylamidase (PLAN) as an indicator of toxicant stress in rainbow trout. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 161-162.

Des études ont été entreprises afin d'évaluer la possibilité de modifier le niveau de l'enzyme protéolytique leucine-amino-naphtylamidase dans le plasma en vue d'évaluer les effets d'intoxications aigues sublétales sur la truite arc-en-ciel. Des prélèvements de sang ont été effectués sur des groupes de poissons de 80 à 100 g, à la suite d'une

exposition, par injection intrapéritoniale ou dans le milieu aqueux, au paraméthylphénol (PMP) ou au tétrachlorure de carbone ($CCl_4$).

La durée de rétention après injection a eu des effets considérables sur l'activité de la LANP chez la truite arc-en-ciel témoin comme chez la truite arc-en-ciel exposée au PMP, la plus forte augmentation survenant après 96 heures. Des relations statistiquement significatives entre dose et activité de LANP on été obtenues chez le poisson ayant subi une injection intrapéritoniale au PMP comme au $CCl_4$. Les poissons ayant reçu du PMP ont montré des niveaux de LANP augmentés de 27 à 63% par rapport aux témoins, 96 heures après injection. Le poisson traité au $CCl_4$ a montré des niveaux de LANP de 38 à 135% plus élevés que chez les témoins, 48 heures après injection. Une concentration du milieu aqueux de 0,028 mM de PMP (0,41 CL50 96 h) a entraîné des accroissements statistiquement significatifs de l'activité de la LANP chez la truite arc-en-ciel après 48, 96 et 192 heures d'exposition. L'activité de la LANP s'est accrue de 38 à 87% par rapport aux témoins. Dans tous les cas, les changements du niveau de protéine plasmatique et du rapport entre le poids du foie et celui du corps reflétaient les modifications de l'activité de la LANP, sans qu'on puisse relever cependant dans les tissus hépatiques de lésion histopathologique significative.

La température et la durée de stockage plasmatique ont eu des effets importants sur l'activité de la LANP chez la truite arc-en-ciel témoin comme chez celle qui avait reçu du PMP. Tandis que la diète s'est montrée un important modificateur de l'activité de la LANP, le sexe n'a eu aucune influence. Nous analyserons les applications possibles de l'activité de la LANP.

# METALLOTHIONEIN AND RESISTANCE TO CADMIUM TOXICITY IN WHITE SUCKERS (CATOSTOMUS COMMERSONI) IMPACTED BY ATMOSPHERIC EMISSIONS FOR A BASE-METAL SMELTER

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KLAVERKAMP, J.F., W.A. MACDONALD, L.J. WESSON, and A. LUTZ. 1985. Metallothionein and resistance to cadmium toxicity in white suckers (Catastomus commersoni) impacted by atmospheric emissions from a base-metal smelter. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 163-164.

Laboratory investigations have demonstrated that exposure to sub-lethal concentrations of Cd, Hg or Zn produced elevated metallothionein (MTN) concentrations and increased resistance to Cd toxicity in white suckers. The purpose of the present study was to extend these laboratory investigations to the field. Suckers from a highly metal-contaminated lake (Hamell), which has received atmospheric deposition of base-metal smelter emissions for over 50 years, were studied and compared to suckers from a less contaminated lake (Thompson) in the Flin Flon, Manitoba area.

In 1980, estimates of MTN concentrations using gel filtration analyses of liver, intestine, and gill from Hamell Lake suckers were 3.6, 4.5, 3.3 times, respectively, those from Thompson Lake suckers. Hamell Lake fish also contained higher total Cu and Zn in liver and intestine. A higher percentage of cellular cytosolic Cu was bound to the crude MTN fraction of liver, intestine and gill of fish from Hamell Lake. In liver from these fish, a higher percentage of cellular cytosolic Zn was bound to the crude MTN fraction.

In 1981, in situ lake toxicity tests were conducted using lethal concentrations of Cd, and gel filtration analyses were extended to include kidney. The toxicity tests demonstrated that suckers from Hamell Lake were up to 2.3 times more resistant to Cd toxicity than Thompson Lake suckers. While some year-to-year variations, which may be due to holding stresses, were observed in the sub-cellular distribution of Cu and Zn, the estimated MTN concentrations in liver, intestine, gill, and kidney of Hamell Lake fish were 2.3 to 4.3 times higher than those estimated for Thompson Lake fish. In crude MTN fractions, there was evidence that Cd displaced Zn and Cu in liver and that Cd only displaced Zn in kidney.

To our knowledge this is the first report showing an association between elevated MTN concentrations and resistance to Cd toxicity in fish exposed to atmospheric deposition of metal emissions in the natural environment. The results will be discussed in relation to the role of MTN and other biochemical mechanisms as compensatory responses to Cd toxicity.

KLAVERKAMP, J.F., W.A. MACDONALD, L.J. WESSON, and A. LUTZ. 1985. Metallothionein and resistance to cadmium toxicity in white suckers (Catastomus commersoni) impacted by atmospheric emissions from a base-metal smelter. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 163-164.

Des recherches en laboratoire ont démontré que l'exposition à des concentrations sublétales de cadmium, de mercure ou de zinc produisait des concentration élevées de métallothionéine (MTN) et un accroissement de la résistance à la toxicité du cadmium chez le meunier noir. L'objet de la présente étude est de transposer sur le terrain les études faites en laboratoire. Les meuniers noirs provenant d'un lac hautement contaminé par des métaux (lac Hamell), recevant depuis plus de 50 ans les émissions atmosphériques d'une fonderie, ont été étudiés et comparés à des meuniers noirs d'un lac moins contaminé (lac Thompson) dans la région de Flin Flon (Manitoba).

En 1980, l'évaluation des concentrations de MTN obtenues par filtration sur gel à partir des tissus du foie, de l'intestin et des branchies des meuniers noirs du lac Hamell s'étaient montrées 3,6, 4,5, 3,3 fois plus élevées que celles des meuniers noirs du lac Thompson. Le foie et l'intestin des poissons du lac Hamell contenaient également une plus forte quantité de cuivre et de zinc. Un pourcentage plus élevé de cuivre cellulaire cytosolique était lié à la fraction brute de MTN du foie, de l'intestin et des branchies du poisson provenant du lac Hamell. Dans le foie de ces poissons, une plus forte proportion de zinc cellulaire cytosolique était liée à la fraction brute de MTN.

En 1981, on a fait des tests de toxicité lacustre in situ, en utilisant des concentrations létales de cadmium, et des analyses de filtration sur gel ont été effectuées sur le rein. Le test de toxicité a montré que les meuniers noirs du lac Hamell étaient jusqu'à 2,3 fois plus résistants à la toxicité du cadmium que ceux du lac Thompson. Tandis que certaines variations annuelles, peut-être dues à des stress de rétention, étaient observées dans la répartition subcellulaire du cuivre et du zinc, les concentrations estimées de MTN dans le foie, l'intestin, les branchies et le rein des poissons du lac Hamell étaient 2,3 à 4,3 fois plus élevées que celles des poissons du lac Thompson. Dans les fractions brutes de MTN, le cadmium déplaçait le zinc et le cuivre dans le foie et le cadmium déplaçait seulement le zinc dans le rein.

A notre connaissance, cette étude est le premier travail qui montre une association entre une concentration élevée de MTN et la résistance à la toxicité du cadmium chez des poissons exposés à des dépôts atmosphériques d'émissions métalliques dans l'environnement naturel. Les résultats seront analysés en fonction du rôle joué par le MTN et d'autres mécanismes biochimiques dans la réaction compensatoire à la toxicité du cadmium.

# INVESTIGATION ON THE MODE OF ACTION OF CYANIDE BY MONITORING VARIOUS PHYSIOLOGICAL PARAMETERS IN RAINBOW TROUT (Salmo gairdneri Richardson) EXPOSED DURING 20 DAYS TO SUBLETHAL CYANIDE LEVELS

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RAYMOND, P. and G. LEDUC. 1985. Investigation on the mode of action of cyanide by monitoring various physiological parameters in rainbow trout (Salmo gairdneri Richardson) exposed during 20 days to sublethal cyanide levels. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 165-166.

The mode of action of cyanide in rainbow trout (Salmo gairdneri, Richardson) during a 20-day exposure to 0.01, 0.02 and 0.03 $mg.L^{-1}$ HCN at 12°C was investigated through a study of the following physiological parameters: liver cytochrome oxidase activity, accumulation of blood plasma thiocyanate, liver glycolytic activity and fish body size.

Results indicate that rainbow trout with a mean weight of 168.5 g ± 30.7 showed a significant reduction ($P<0.001$) of 80% in liver cytochrome oxidase activity within 24 hr. of exposure to all three concentrations of HCN. Rainbow trout with a mean weight of 32.3 g ± 4.9 showed a significant ($P<0.001$) reduction in liver cytochrome oxidase activity of 60% of the control response and their response relative to the larger fish was more gradual and dose-related. For both fish sizes examined, the control cytochrome oxidase activity differed, the larger fish having a greater activity per mg. of protein. The minimal activity, however, in fish exposed to any of the three cyanide concentrations was almost identical for both fish sizes.

Sublethal concentrations of cyanide significantly reduced rainbow trout liver glycogen levels with regard to length of exposure ($P<0.001$) and cyanide concentrations ($P<0.01$). After 20 days of exposure, all of the cyanide-exposed fish groups with the exception of the 0.03 $mg.L^{-1}$ HCN group returned to glycogen levels comparable to that of the control.

Compared with the controls, blood plasma thiocyanate levels of rainbow trout exposed to cyanide throughout the 20-day period were significantly increased both by cyanide concentration and duration of exposure; however, the pattern of thiocyanate accumulation varied between experiments, probably because of differences related to fish size and/or season.

The significance of these results with respect to the mode of action of cyanide will be discussed in relation to current knowledge of sublethal toxicity of cyanide and thiocyanate.

RAYMOND, P. and G. LEDUC. 1985. Investigation on the mode of action of cyanide by monitoring various physiological parameters in rainbow trout (Salmo gairdneri Richardson) exposed during 20 days to sublethal cyanide levels. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 165-166.

Le mode d'action des cyanures chez la truite arc-en-ciel (Salmo gairdneri Richardson) pendant une exposition de 20 jours à des doses de 0,01, 0,02 et 0,04 mg/L de HCN à 12°C a été étudié en surveillant les paramètres physiologiques suivants: activité de la cytochrome oxydase du foie, accumulation du thiocyanate plasmatique, activité glycolytique du foie, et taille du poisson.

Les résultats indiquent que chez la truite arc-enc-ciel d'un poids moyen de 168,5 g ± 30,7 il y a eu une réduction significative ($P < 0{,}001$) de 80% de l'activité de la cytochrome oxidase du foie en 24 heures d'exposition, aux trois concentrations de HCN. Les truites arc-en-ciel d'un poids moyen de 32,3 g ± 4,9 ont montré une réduction significative ($P < 0{,}001$) de l'activité de la cytochrome oxydase du foie, se chiffrant à 60% de la réaction des sujets témoins, et leur réaction par rapport à celle des grands poissons a été plus progressive et dépendait davantage de la dose. Chez les poissons des deux tailles examinées, l'activité de la cytochrome oxydase témoin a varié, les gros poissons montrant une plus grande activité par milligramme de protéine. Cependant, l'activité minimale chez les poissons exposés à l'une quelconque des trois concentrations de cyanure a été presque identique pour les deux tailles de poisson.

Les concentrations sublétales de cyanure ont réduit de façon significative les niveaux de glycogène hépatique des truites arc-en-ciel compte tenu de la longueur de l'exposition ($P < 0{,}01$). Après 20 jours d'exposition, la totalité des groupes de poissons exposés au cyanure, à l'exception de ceux qui avaient été exposés à 0,03 $mg/L^{-1}$ de HCN, sont revenus à des niveaux de glycogène comparables à ceux des animaux témoins.

Si on établit une comparaison avec les témoins, les niveaux de thiocyanate plasmatique des truites arc-en-ciel exposées au cyanure pendant la période de 20 jours ont augmenté de façon significative à la fois par la concentration en cyanure et par la durée de l'exposition; cependant, l'accumulation de thiocyanate variait d'une expérience à l'autre, probablement en raison des différences de taille des poissons ou de la saison.

La valeur des résultats en ce qui concerne le mode d'action des cyanures sera analysées en fonction des connaissances actuelles sur la toxicité sublétale du cyanure et du thiocyanate.

# ASSESSMENT OF PHYSIOLOGICAL STRESS TO RAINBOW TROUT USING ATP MEASUREMENTS WHILE CONDUCTING ACUTE LETHAL TOXICITY TESTING OF INDUSTRIAL EFFLUENTS

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TROTTIER, B. and C. BLAISE. 1985. Assessment of physiological stress to rainbow trout using ATP measurements while conducting acute lethal toxicity testing of industrial effluents. Can. Tech. Rep. Fish. Aquatic. Sci. 1368: p. 167.

A procedure was developed to assess physiological stress to rainbow trout as measured by ATP while conducting standardized 96 - hour LC50 acute lethal toxicity tests of industrial effluents. By a method which is described herein, nucleotide levels were eventually measured in muscle tissue samples taken from control fish and test fish survivors which were immediately anesthetized following completion of the standardized bioassay. An objective of this work attempted to identify the lowest active concentration, for each effluent tested, at which stress was observable. Current results of this ongoing work as well as the potential uses and applications of this methodology for environmental protection activities are discussed.

TROTTIER, B. and C. BLAISE. 1985. Assessment of physiological stress to rainbow trout using ATP measurements while conducting acute lethal toxicity testing of industrial effluents. Can. Tech. Rep. Fish. Aquatic. Sci. 1368: p. 167.

Une méthode a été mise au point afin d'évaluer le stress physiologique chez la truite arc-en-ciel par mesure du niveau d'ATP en cours d'essai normalisé de toxicité létale aigue (CL50 - 96 heures) d'effluents industriels. Au moyen d'une méthode décrite dans le présent article, nous avons finalement mesuré les niveaux de nucléotide dans des échantillons de tissu musculaire prélevés sur des poissons témoins et sur des poissons survivants, qui ont été immédiatement anesthésiés dès l'achèvement de l'essai biologique normalisé. Un des objets de l'étude était de tenter de déterminer la concentration active la plus basse possible, pour chaque effluent testé, à laquelle on pouvait observer un stress. Nous analysons ensuite les résultats de cette étude en cours, ainsi que les utilisations et applications possibles de cette méthodologie pour des activités de protection de l'environnement.

# METAL TOXICOLOGY

Scott MacKnight, Chairman

# HEAVY METAL INTERACTION IN JUVENILE AMERICAN LOBSTER (*HOMARUS AMERICANUS*)

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CHOU, C.L., J.F. UTHE, J.D. CASTELL, and J.C. KEAN. 1985. Heavy metal interaction in juvenile American lobster (*Homarus americanus*). Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 169-182.

Investigations were carried out on the effect of dietary cadmium or dietary protein source (casein or Crab) on survival, growth, digestive gland size, trace metal uptake (zinc, copper, cadmium and silver) by the digestive gland and tail muscle, and metal-metal interactions in juvenile lobster fed for seventeen weeks. The addition of as much as 45 mg Cd/Kg diet did not affect survival but had a slight effect on growth. Growth on a crab-based diet was slightly better than growth on a casein-based diet. Lobsters fed the casein-based diet had relatively larger digestive glands than those of wild or crab-fed lobsters. Cd was linearly taken up by the digestive gland over the dietary Cd range. Much lower uptake was observed in the muscle tissue and the uptake was limited, being described by a semi-logarithmic relationship which suggests that there is a rate limited uptake. The absence of ascorbate from the diet increased the digestive gland uptake of Cd but had no effect on any other parameter studied. Cd in the crab-based diet appeared to be more readily taken up by the digestive gland than was inorganic Cd in the casein-based diet. Zn levels in the digestive gland demonstrated a complex response to the presence of Cd in the crab diet as did Cu and Ag in both digestive gland and tail muscle. Muscle Zn levels were unaffected by any dietary manipulations suggesting that muscle Zn levels are under biological control. The addition of Cd to the crab-based diet destroyed the highly significant relationship between tissue Ag and Cu which has been observed in wild animals. Cu in the crab-based diet was taken up by the digestive gland much more efficiently than Cu in the casein-based diet.

CHOU, C.L., J.F. UTHE, J.D. CASTELL, and J.C. KEAN. 1985. Heavy metal interaction in juvenile American lobster (*Homarus americanus*). Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 169-182.

On a fait des études sur les effets du cadmium alimentaire ou de la source de protéine alimentaire (caséine ou crabe) sur la survie, la croissance, la dimension des glandes digestives, la fixation des métaux à l'état de trace (zinc, cuivre, cadmium et argent) par la glande digestive et le muscle de la queue et les interactions métal-métal chez de jeunes homards nourris pendant 17 semaines. L'addition de 45 mg de cadmium par kilogramme n'a pas eu d'influence sur le taux de survie, mais a eu un léger effet sur la croissance. Avec une diète à base de crabe, la croissance a été légèrement plus importante qu'avec une diète à base de caséine. Avec la diète à base de caséine, les

homards ont présenté des glandes digestives relativement plus grandes que les homards observés sauvages ou recevant une nourriture à base de crabe. Le cadmium a été fixé de façon linéaire par la glande digestive au-delà de la limite du cadmium diététique. La fixation dans le tissu musculaire a été très inférieure et limitée, pouvant se décrire selon une relation semi-logarithmique suggérant une fixation limitée par le taux. L'absence d'ascorbate dans le régime a augmenté la fixation du cadmium par la glande digestive, mais n'a pas eu d'effet sur les autres paramètres étudiés. Chez les homards nourris de crabe, le cadmium était fixé plus rapidement par la glande digestive que le cadmium inorganique chez les homards nourris à la caséine. Les niveaux de zinc dans la glande digestive ont montré une réaction complexe à la présence de cadmium dans le régime à base de crabe, de même que le cuivre et l'argent dans la glande digestive et le muscle de la queue. Les niveaux de zinc dans le muscle n'ont pas été influencés par les manipulations diététiques, semblant indiquer que les niveaux musculaires de zinc sont sous contrôle biologique. L'addition de cadmium au régime à base de crabe a détruit la relation hautement significative entre l'argent et le cuivre tissulaires observée chez les animaux en milieu naturel. Dans le régime à base de crabe, le cuivre a été fixé par la glande digestive de façon beaucoup plus efficace que dans le régime à base de caséine.

## INTRODUCTION

Shellfish are known to accumulate high levels of trace metals in their tissues, particularly the digestive gland (Hepatopancreas). In the American lobster (*Homarus americanus*) extremely high levels of Cadmium (Cd) have been documented in digestive glands from animals captured near a lead smelter (Uthe et al. 1980, 1981) without apparent effect on the well-being of the animals. The high level of Cd in lobster and the lack of sub-lethal effects make it tempting to postulate a biochemical role for Cd in this species. We have investigated the uptake of Cd by digestive gland and tail muscle of juvenile lobsters fed either a casein or crab-based diet fortified with various levels of inorganic Cd. The effects of these dietary manipulations upon levels of copper (Cu), silver (Ag), and zinc (Zn) in the two tissues were also determined.

### Materials and Methods

A total of 60 juvenile lobsters were used for each dietary treatment. Animals were housed individually and fed *ad libitum* thrice daily, five days a week with a single feeding during the weekend. Excess food and fecal matter were removed prior to each feeding. The dietary regimes are given in Table 1. After 17 weeks of feeding the surviving animals were starved for 48 hours; digestive gland and tail muscle tissue were removed 3-5 animals and pools of each tissue type prepared. Weight gain and survival data were collected over the course of the feeding trial. Levels of Cd, Cu, and Zn in the digestive gland and Cu and Zn in the tail muscle pools were determined by flame atomic absorption spectrophotometry while digestive gland Ag levels and tail muscle Cd and Ag levels were determined by graphite furnace atomic absorption spectrophotometry.

TABLE 1 DIETARY COMPOSITIONS

| Constituent[1] | % By Weight |
|---|---|
| Casein (or crab) | 50 |
| Gelatin | 10 |
| Corn Starch | 5 |
| Cellulose | 8.8 |
| Mineral Mix | 5 |
| Lecithin | 6 |
| Cod Liver Oil | 10 |
| Glucosamine | 1 |
| Cholesterol | 1 |
| Vitamins (0.12% Ascorbate) | 2.2 |

TABLE 1 DIETARY COMPOSITIONS (Cont'd)

| Diet | Total Cd$^{2}$ | Added Cd | Cu$^{2}$ | Zn$^{2}$ | Ag$^{2}$ | (All in mg/kg) |
|---|---|---|---|---|---|---|
| Casein-$^{1}$ | 0 | 0 | 16.7 | 0 | 0 | |
| Casein-$^{2}$ | 2.5 | 2.5 | 16.7 | 60 | 0 | |
| Crab-$^{3}$ | 7.5 | 2.5 | 76.7 | 155 | 4.10 | |
| Crab-$^{4}$ | 10.0 | 5.0 | 76.7 | 155 | 4.10 | |
| Crab-$^{5}$ | 15.0 | 10 | 76.7 | 155 | 4.10 | |
| Crab-$^{6}$ | 25.0 | 20 | 76.7 | 155 | 4.10 | |
| Crab-$^{7}$ | 45.0 | 40 | 76.7 | 155 | 4.10 | |
| Crab-$^{8}$ | 25.0 | 20 | 76.7 | 155 | 4.10 | (No ascorbate) |

1 - Composition and formulation described in Chou et al. 1981.
2 - Concentration determined by chemical analysis.

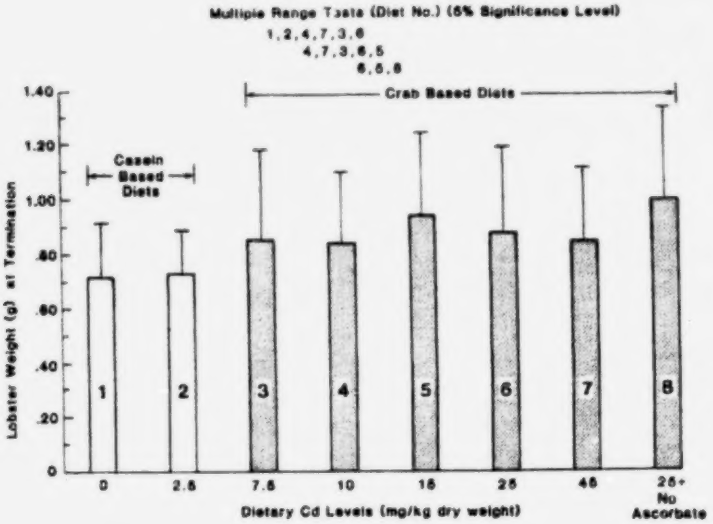

FIGURE 1 FINAL MEAN WEIGHTS OF JUVENILE LOBSTERS FED EITHER CRAB OR CASEIN-BASED DIETS WITH VARIOUS LEVELS OF ADDED CD. (STANDARD DEVIATION BARS ARE SHOWN)

## CONCLUSIONS

1. Lobsters fed a crab-based diet generally grew better than animals fed a casein-based diet although there was no significant weight increase in those animals fed crab-based diets with lower levels of Cd compared to the animals fed a casein-based diet.

2. Ascorbate does not appear to be required for satisfactory growth of lobsters.

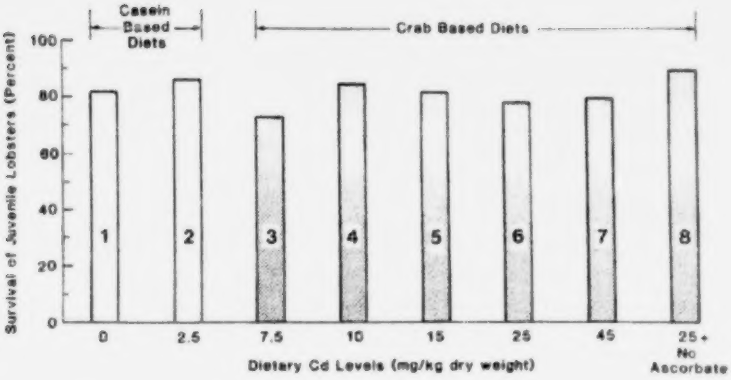

FIGURE 2 SURVIVAL OF JUVENILE LOBSTERS FED FOR 17 WEEKS ON EITHER CRAB-OR A CASEIN-BASED DIET WITH VARYING LEVELS OF CD

## CONCLUSIONS

1. Juvenile lobsters fed for 17 weeks on either a casein-or a crab-based diet spiked with varying amounts of added Cd did not show any significant effects on survival.

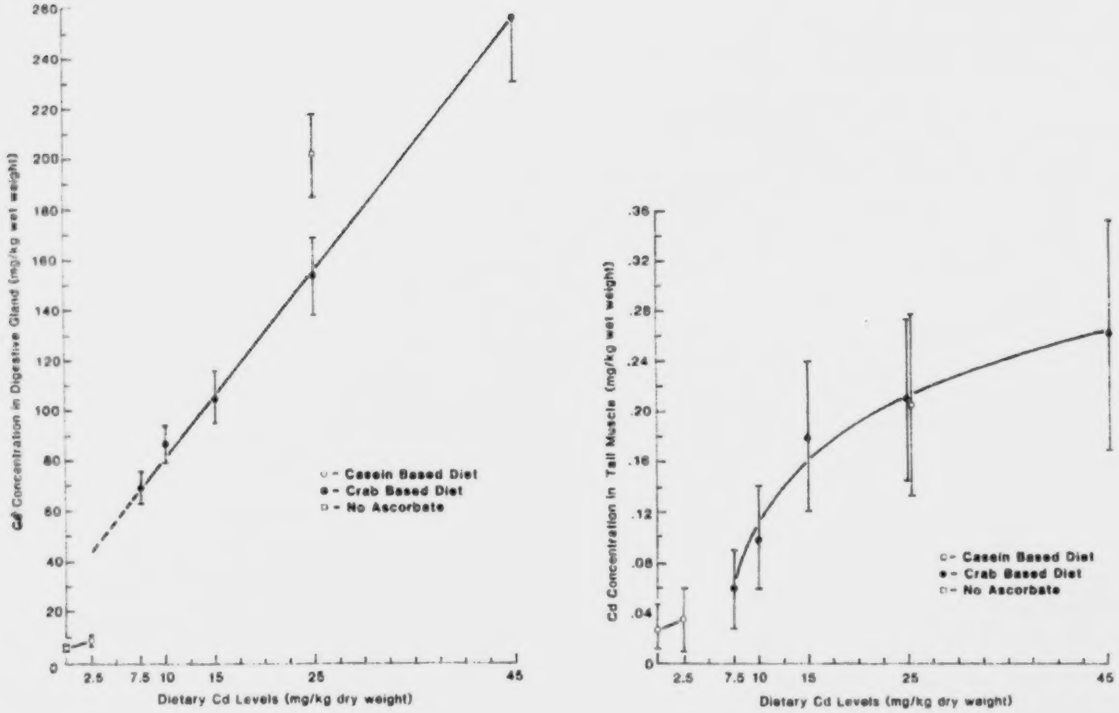

FIGURE 3 UPTAKE OF CD BY DIGESTIVE GLAND AND TAIL MUSCLE OF LOBSTER

## CONCLUSIONS

1. Cd levels in both tissues increased with increasing Cd dietary levels. Tail muscle Cd levels were much lower than digestive gland levels.

2. The response of the tail muscle Cd to dietary Cd did not increase as much at the higher dietary levels as at the lower levels suggesting that there is some limiting factor in muscle Cd uptake.

3. Cd when fed in a crab-based diet (naturally containing 5 mg Cd/kg) was better taken up by the digestive gland than Cd fed in a casein-based diet (in Figure 3 the crab diet response is extrapolated down to the equivalent casein diet).

4. The absence of dietary ascorbate resulted in an increased uptake of dietary Cd by the digestive gland but not by the muscle tissue.

5. The uptake of Cd by the digestive gland from crab-based diets can be described by the equation:

   Mean (Cd) digestive gland = 57.13 + 12.46 (Cd) diet.

   The Coefficient of Determination was 0.999.

6. The uptake of Cd by the tail muscle from crab-based diets can be described by the equation:

   Mean (Cd) tail muscle = 0.059 + 0.074 log (Cd) diet.

   The Coefficient of Determination was 0.983.

7. Feeding crab-based diets with 2.5 mg/kg added to Cd to juvenile lobsters for 17 weeks resulted in Cd levels of 70 mg/kg for the digestive gland and 0.06 mg/kg for the tail muscle. The Cd level in the digestive gland is much higher than levels observed in uncontaminated wild lobster (mean level 19 mg/kg) (Uthe et al. 1980), while the Cd level in the tail muscle is quite similar to Cd levels in lobster found in less Cd-contaminated Belldune Harbour, New Brunswick (Uthe et al. 1981).

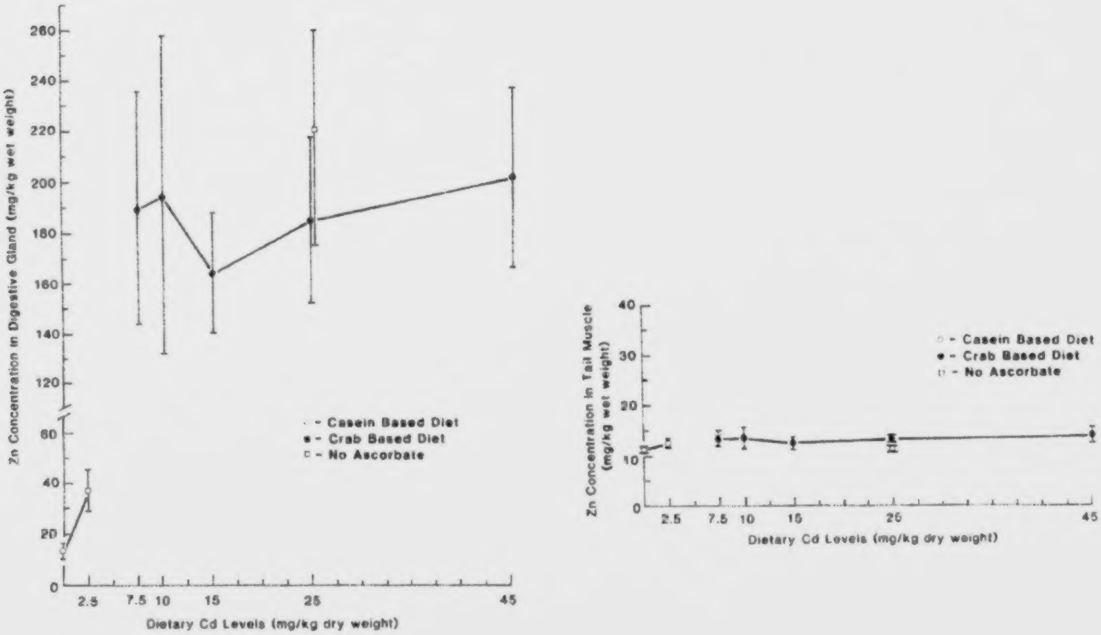

FIGURE 4 UPTAKE OF ZN BY DIGESTIVE GLAND AND TAIL MUSCLE OF LOBSTER

## CONCLUSIONS

1. A greater uptake of Zn (approximately 5 times) by the digestive gland was observed in animals fed crab-based diets than those fed casein-based diets. This is probably due to the higher level of Zn present in the crab-based diet (approximately 3 times) than in the casein-based diet.

2. There was no significant difference in Zn uptake by the muscle tissue from animals fed either crab-or casein-based diets.

3. Varying dietary levels of Cd had no effect on Zn levels in tail muscle and appeared to decrease digestive gland Zn levels at the lower dietary levels of Cd then increase them with higher dietary levels of Cd.

4. The absence of ascorbate had no effect on Zn levels in either tissue.

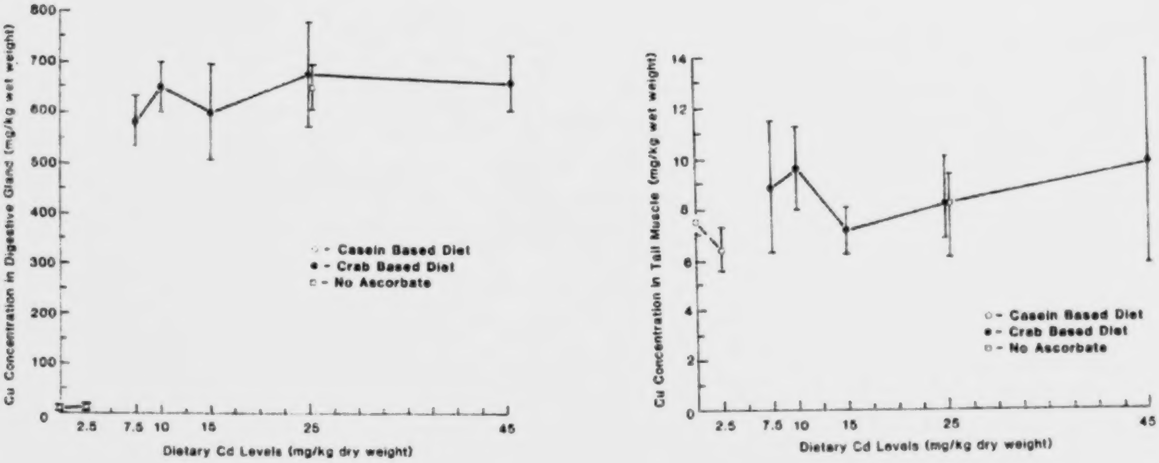

FIGURE 5 UPTAKE OF CU BY DIGESTIVE GLAND AND TAIL MUSCLE OF LOBSTER

## CONCLUSIONS

1. As found with Zn, a markedly greater uptake of Cu by the digestive gland was observed in animals fed crab-based diets (approximately 50 times) than those fed casein-based diets. This is probably due to both bioavailability and the higher level of Cu present in the crab-based diet (approximately 5 times) than in the casein-based diet.

2. There was no significant difference in Cu uptake by the muscle tissue from animals fed either a crab-or a casein-based diet.

3. Varying dietary levels of Cd appeared to decrease digestive gland Cu levels at the lower dietary levels of Cd then increase them with higher dietary levels of Cd.

4. The absence of ascorbate had no effect on Cu levels in either tissue.

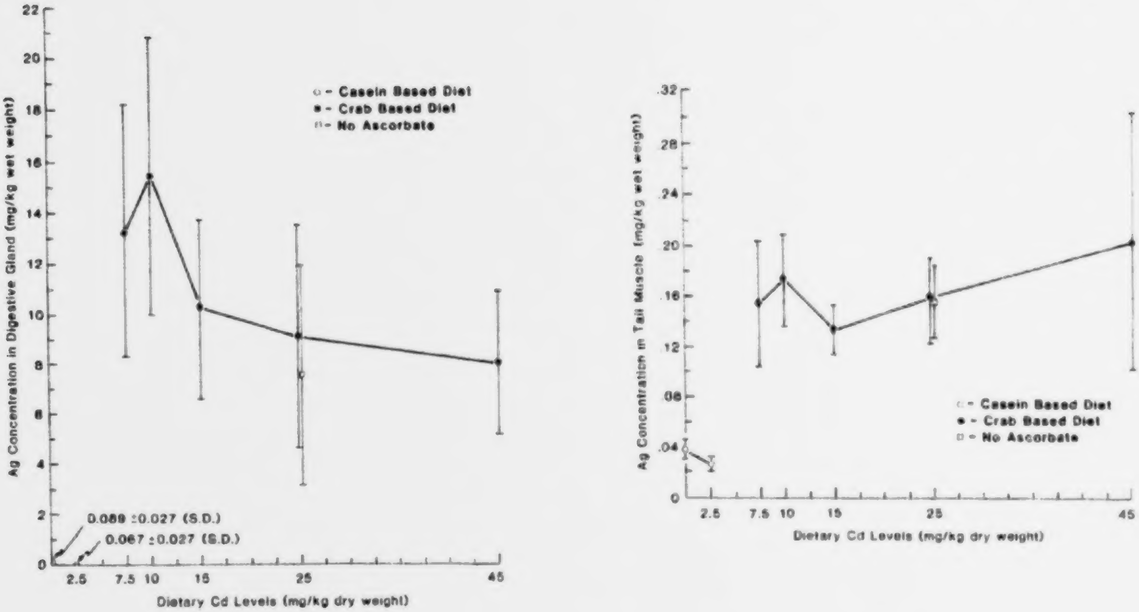

FIGURE 6 UPTAKE OF AG BY DIGESTIVE GLAND AND TAIL MUSCLE OF LOBSTER

## CONCLUSIONS

1. Higher levels of Ag were found in both digestive gland and tail muscle of lobsters fed a crab-based diet than in animals fed a casein-based diet. This is due to the higher level of Ag in the crab-based diet.

2. The uptake of Ag by the digestive gland generally decreased as the level of Cd in the diet increased, although a small increase was observed at the lower levels of added Cd.

3. The uptake of Ag by the tail muscle generally increased as the level of Cd in the diet increased, although a small increase then a decrease was noted with the dietary Cd levels between 7.5 mg Cd/kg and 15 mg Cd/kg.

4. The uptake of Ag by both tissues was not affected by the absence of ascorbate in the diet.

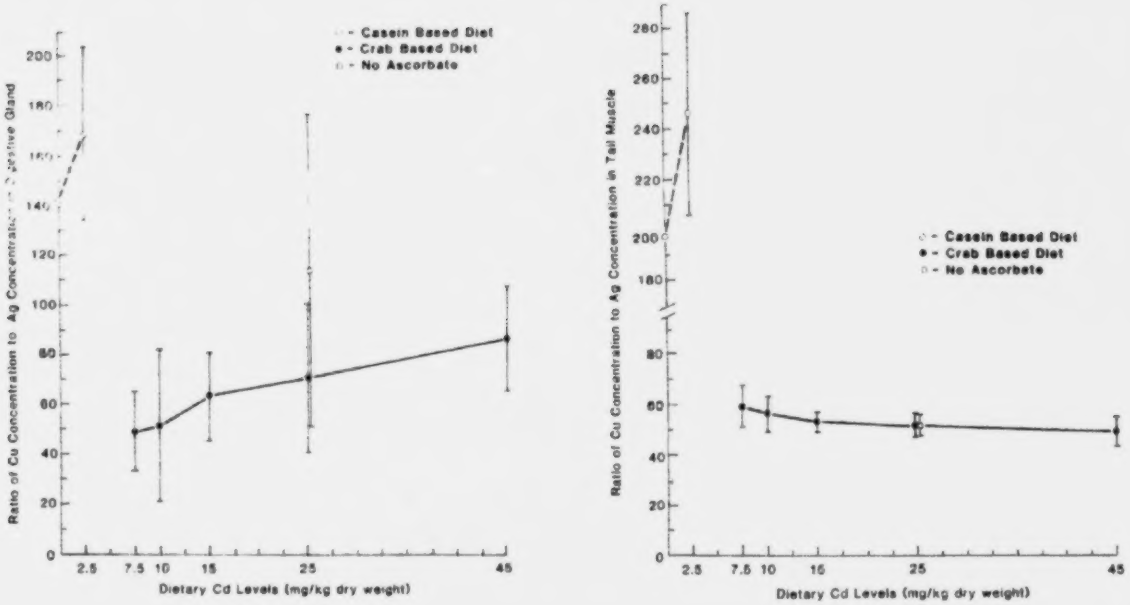

FIGURE 7 THE EFFECT OF PROTEIN SOURCE AND VARYING DIETARY CD LEVELS ON THE RATIO OF CU TO AG CONCENTRATIONS IN LOBSTER DIGESTIVE GLAND AND TAIL MUSCLE

## CONCLUSIONS

1. Markedly higher Cu/Ag ratios were present in tissues of lobster fed a casein-based diet than in those fed a crab-based diet. The casein Cu/Ag ratios were significantly higher than the ratios observed in natural populations of adult lobsters (Chou and Uthe 1978).
2. The Cu/Ag ratio in digestive gland increased significantly with increasing levels of dietary Cd in the crab-based diet.
3. The Cu/Ag ratio in tail muscle decreased slightly with increasing levels of Cd in the crab-based diet.
4. Ascorbate did not affect Cu/Ag ratios in either tissue.
5. Addition of Cd to the casein-based diet increased the Cu/Ag ratio in both tissues.

In natural populations of adult lobsters there is a significant relationship between the concentration of Cu and Ag in the digestive gland (Chou and Uthe 1978). The same is true for muscle tissue. The correlation coefficients ranged from 0.742-0.949 in lobster digestive gland for 5 different sampling locations. We have investigated the Cu/Ag relationships in lobsters from each feeding regime (Table 2).

TABLE 2 COEFFICIENTS OF DETERMINATION FOR THE RELATIONSHIP BETWEEN TISSUE LEVELS OF CU AND AG IN JUVENILE LOBSTERS FED VARIOUS DIETS

| Diet | Digestive Gland | Tail Muscle |
|---|---|---|
| Casein-[1] | 0.817 | 0.806 |
| Casein-[2] | 0.860 | 0.723 |
| Crab-[3] | 0.516 | 0.862 |
| Crab-[4] | 0.230 | 0.930 |
| Crab-[5] | 0.058 | 0.801 |
| Crab-[6] | 0.252 | 0.820 |
| Crab-[7] | 0.092 | 0.956 |
| Crab-[8] (No Ascorbate) | 0.004 | 0.753 |

A highly significant relationship was found in the tail muscle with all dietary regimes while in the digestive gland the relationship was highly significant only for the casein-based diets. The addition of more than 2.5 mg Cd/kg diet resulted in the destruction of the relationship.

## DISCUSSION

The results of this study show that lobster growth and survival were not affected by dietary Cd levels as great as 25 mg/kg. Even with a dietary Cd level of 45 mg/kg there was no effect on survival and a barely significant effect upon weight gain. Dietary Cd was accumulated in the digestive gland and showed a linear relationship to the dietary Cd level. This implies that the maximum rate of Cd uptake by this gland had not been reached in spite of a glandular level in excess of 250 mg Cd/kg being achieved with the diet containing 45 mg Cd/kg. This is not surprising since apparently healthy lobsters with digestive gland Cd concentrations in excess of 400 mg Cd/kg have been captured in a contaminated area (Uthe et al. 1981). Uptake of Cd by the tail muscle was much less than uptake by the digestive gland and it appears that the entrance of Cd into the muscle is strictly limited since the response curve levels off at the higher dietary levels of Cd.

Both digestive gland and tail muscle levels of Cd of lobsters fed a crab-based diet with the addition of 2.5 mg/kg inorganic Cd were higher than levels observed in wild populations of lobster (Uthe et al. 1980). Use of such a diet in either experimental or commercial rearing of lobster must take this into account since experimental animals with higher than usual Cd levels may show subtle sub-lethal effects and commercial animals with such Cd levels may be banned from commercial sales by health agencies.

Tissue levels of Zn, Cu, and Ag all showed response to the various diets, but the responses were different from the patterns observed with the tissue levels of Cd. The levels of Zn in the tail muscle were unaffected by either diet (different Zn levels) or by the addition of dietary Cd. The refractory nature of the Zn levels in the tail muscle likely means that such levels are very carefully controlled by the animals and one would not expect significant changes in the Zn levels until the animals are in a pathological state.

The digestive gland levels of Zn and the tissue levels of Cu and Ag in both tissues showed a complex response to the diet protein source and the Cd dietary level. In general, probably due to the higher levels of Zn, Cu, and Ag in the crab-based diets these tissue levels were higher than those found with the casein-based diets. The addition of 60 mg Zn/kg to the casein-based diet resulted in an increase in the digestive gland level of Zn but the increase had been raised to the 155 mg Zn/kg present in the crab diet. This implies that Zn present in the crab-based diet is more bioavailable than inorganic Zn in the casein-based diet. Similarly, the uptake of Cu by the digestive gland from the crab-based diet (76.7 mg Cu/kg) was much greater than expected from an equivalent Cu level in the casein-based diet. The Cu levels in the muscle were not significantly affected by the protein base of the diet. These results imply that Cu naturally present in the crab used in diet formulation is more bioavailable to lobster than if an equivalent amount of inorganic Cu had been added to the casein-based diet. The presence of naturally occurring levels of Ag in the crab-based diets resulted in higher Ag levels in the digestive gland and the tail muscle compared with the casein-based diets which contained immeasurable levels of Ag. The positive response of Ag levels in the muscle differs from those of Cu and Zn although this may simply be a reflection of the extremely low levels of Ag in the casein-based diet.

The effect of dietary Cd upon tissue levels of other metals is very complex. There appears to be an increased tissue uptake of the three metals, Ag, Cu, and Zn, with Cd dietary levels of between 7.5 and 10 mg/kg, then a decrease between 10 and 15 mg/kg then some increase in tissue levels with further increases in dietary Cd with the exception of the response of Ag in the digestive gland. We are not able to interpret these complex results, other than to say that such a complex response has been observed by us in an earlier study of the response of tissue Ag and Cu levels to various amounts of Ag and Cu in a casein-based diet (Chou et al. 1981). The results reported here are all based upon tissue concentrations and it may be postulated that the complex responses in metal concentrations are due to changes in the size (mass) of the muscle or the digestive gland. We have investigated the weights of the digestive glands from the various dietary regimes as well as the percent ratio of digestive gland weight to total animal weight. Digestive gland weights did not differ with the Cd modified diets. However, animals fed casein-based diets had a larger-than-expected mean digestive gland weight of 6.33 ± 0.52% of body weight while those fed a crab-based diet had a mean digestive gland weight of 5.39 ± 0.67% of body weight. In studies of wild adult lobsters a ratio of 5.22 ± 0.12 has been reported (Stewart et al. 1967).

Wild lobsters demonstrate a high correlation between tissue Cu and Ag levels, the linear relationship being described by a log-log equation. The ratio of the concentrations of Cu to Ag (Figure 7) as affected more by the diet protein source than the addition of Cd to the diets. A high Cu/Ag ratio was observed with the basal casein diet, which increased when both Zn and Cd was added to the diet. This high ratio was likely due to the lack of Ag in the casein-based diet. A much lower Cu/Ag ratio was found with crab-based diets which contained an appreciable amount of Ag. The addition of Cd to the crab-based diet resulted in the Cu/Ag ratio increasing in the digestive gland with increasing dietary Cd and decreasing in the tail muscle. Such changes, particularly in the tail muscle suggests that the translocation and uptake of these two metals by the tail muscle is not mediated by a common carrier protein.

Cd, added to the crab-based diet destroyed the tight relationship (correlation) between Cu and Ag in the digestive gland that was observed in both wild adult lobsters and those fed the casein-based diet. The Cu/Ag relationship suggests that Cu and Ag have some type of biochemical interaction, such an interaction being concentration dependent. The lack of a significant correlation between these two elements in the digestive gland suggests that the uptake of Ag and Cu by the digestive gland occurs independently. The maintenance of the relationship in the tail muscle suggests that some type of biochemical control on muscle levels of these elements is exerted by the animal.

With the exception of an increase in the uptake of Cd by the digestive gland, the absence of ascorbate in the diet did not affect growth, survival, or the level of Zn, Cu or Ag in the two tissues or the level of Cd in the tail muscle. The increased uptake of Cd by the digestive gland in the absence of ascorbate may have resulted from increased leaching of Cd from the food into the surrounding water in the presence of ascorbate or a direct effect of ascorbate on the uptake of Cd.

Overall, the results of these studies have shown that the uptake and tissue levels of dietary trace metals are not simply predicted by the concentration of each element in the diet. Marked interactions occur among the trace elements themselves and between trace metals and other dietary constituents such as the source of the protein used in formulating the diet. Toxic effects of dietary trace metals are difficult to predict from either dietary concentrations or, indeed, tissue concentrations of the metal. For example, we have shown that dietary Ag and Cu interact in such a manner that there is an optimum ratio of these two trace metals. Dietary levels above or below this optimum resulted in decreased normalized biomass (growth x survival) (Chou et al. 1981). It is obvious from these studies that toxicological experiments within the laboratory must take nutritional characteristics of both the animal and the diet into account when interpreting their results or extrapolating their findings to field conditions.

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# WATER QUALITY AND HEAVY METAL CONTAMINANTS IN THE COASTAL WATERS OF NEW BRUNSWICK AND PRINCE EDWARD ISLAND

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LAKSHMINARAYANA, J.S.S. and S.D. JONNAVITHULA. 1985. Water quality and heavy metal contaminants in the coastal waters of New Brunswick and Prince Edward Island. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 183-197.

Water quality characteristics along with major and minor heavy metals of some of the coastal waters of New Brunswick and Prince Edward Island were monitored at different times during 1971-1982. Data from these surveys have shown distinct variations and indicate that these coastal waters are getting polluted. There are several environmental changes in the past, suggesting a rapidly increasing deterioration of environmental quality. The regional differences in the distribution of the contaminants are usually the result of industrial, fishery, agricultural, recreational and other activities of man besides the geochemical and anthropogenic contamination. Oysters collected from different sites of New Brunswick showed higher concentrations of trace metals than in the water samples. Importance of water quality in coastal mariculture is indicated.

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Les caractéristiques de qualité de l'eau, ainsi que les métaux lourds majeurs et mineurs de certaines des eaux côtières du Nouveau-Brunswick et de l'Île-du-Prince-Édouard ont été surveillées à diverses reprises entre 1971 et 1982. Les données obtenues à partir de ces études ont montré des variations distinctes tout en indiquant que ces eaux côtières sont en train de se polluer. Au cours des années passées, il y a eu plusieurs modifications environnementales indiquant une détérioration rapide de la qualité de l'environnement. Les différences régionales dans la répartition des contaminants sont généralement le résultat d'activités halieutiques, industrielles, agricoles, récréatives et autres, en plus de la contamination géochimique et anthropogénique. Des huîtres recueillies à différents endroits du Nouveau-Brunswick ont montré de plus fortes concentrations de métaux à l'état de trace que dans les échantillons d'eau. L'importance de la qualité de l'eau en mariculture côtière est aussi signalée.

## INTRODUCTION

Man has contributed from his diverse activities to the flux of heavy metal and other pollutants from land to sea. The coastal waters generally decrease these pollutants by natural dilution. However, marine organisms remove these pollutants, particularly heavy metals, by precipitation, absorption and adsorption (Bryan, 1971). Distribution of heavy metals in British coastal waters (Preston, 1973) and mercury in fish and food (Bligh, 1971) were noted. There are more systematic time-series data for the open oceans and distant fisheries than for the much more readily accessible coastal waters and estuaries. Significant changes in pollution of some of the marginal coastal waters of the Atlantic region have been noted (Bartlet, 1971; Lakshminarayana and Jean-Pierre, 1975; Machell, 1976). Bewers et al (1974) gave an account of the trace metals in the Gulf of St. Lawrence. Continual monitoring of the coastal water quality will help in understanding and to develop steps to prevent and control any pollution. We report in this paper results of monitoring the water quality and heavy metal contents of some of the coastal waters of New Brunswick and Prince Edward Island.

### Material and Methods

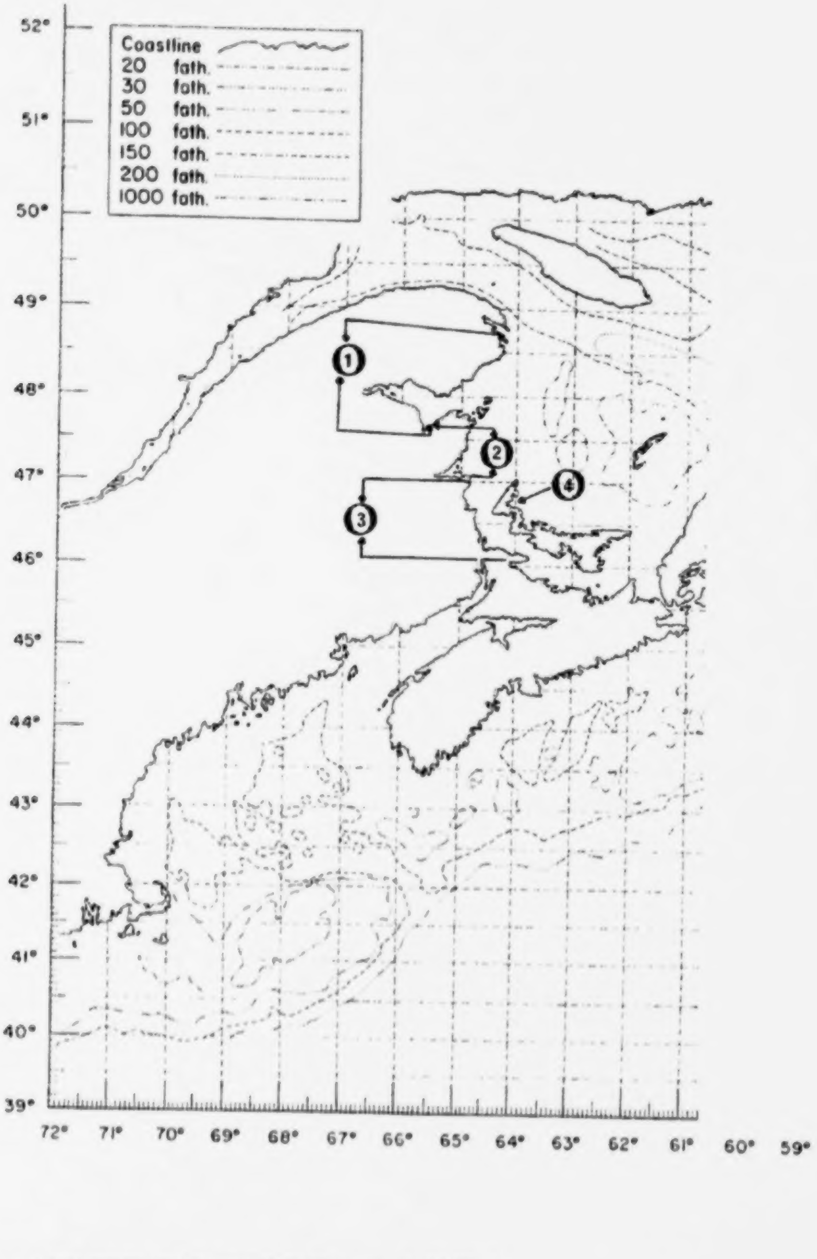
The study area, Northeastern New Brunswick and Prince Edward Island, is divided into four regions (Fig. 1) for purposes of convenience based on the period and duration of study; Region 1 covers Jacket-River, Belledune, Petit Rocher, Nigadoo, Beresford, Bathurst, Anse Blue, Maisonnette and up to Caraquet. From Caraquet region 2 starts encompassing the coastal waters up to Baie du Vin. Proceeding in the same direction Northumberland Strait coastal waters were covered up to Cap Pelé forming the region 3. Malpeque Bay, P.E.I., represents the region 4. Where 22 sampling stations (Fig. 2) were occupied and from all the stations a total of 224 collections were made at various periods from June 1973 to July 1974 as follows:

(a) samplings for a whole year at stations N1, N3 & N6
(b) summer and autumn samplings at stations N2, N4 and N5
(c) summer samplings at stations M1 to M16.

Surveys carried out with the help of students from time to time and some of the results from their reports (Basque et al, 1974; Bourque et al, 1974; Blanchard et al, 1975) are included in this paper. Methods for the collection of samples and analyses were described in Lakshminarayana and Jean-Pierre, 1975; Lakshminarayana, 1976; Lakshminarayana and Bourque, 1979 and Jonnavithula, 1980.

## RESULTS AND DISCUSSION

Summary of the results are given in Tables 1-6. Region 1 supports industries of ABC Packers, Booth Fisheries, Produits Bell Baie, Carapec, East Coast Smelting and Chemical Co. Ltd., Blue Cove Packing Company, Consolidated Bathurst and Belledune Fertilizer. Some people of the region are connected with the exploitation of the peat moss, mining (Brunswick and Nigadoo Mines), piggery (at Burnsville) and other municipal and industrial operations. From 1970 there was a pronounced fall in the quantity of fish landed although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20 fath.
30 fath.
50 fath.
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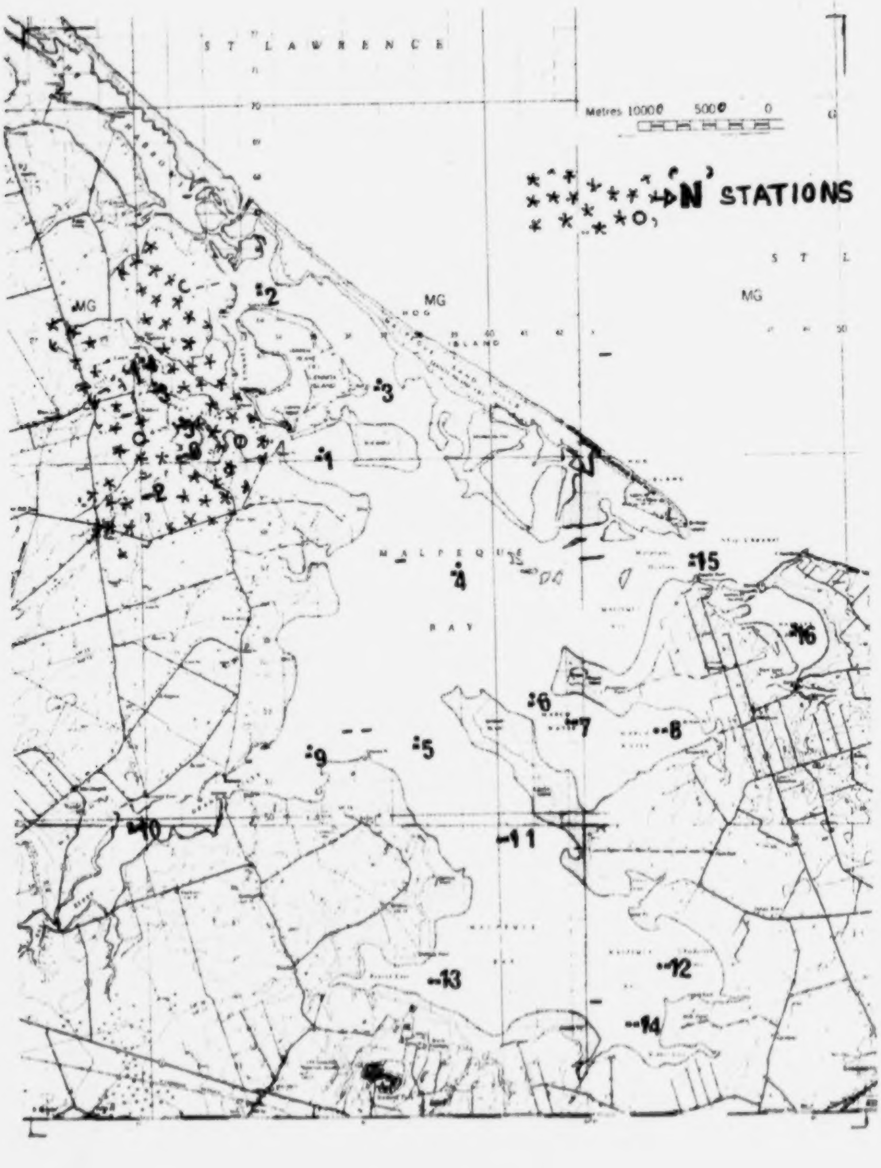
FIGURE 1 MONITORING REGIONS 1, 2, 3 AND 4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16

FIGURE 2 MALPEQUE BAY SAMPLING STATIONS N1 TO N6 & M1 TO M16

TABLE 1 COASTAL WATER QUALITY OF REGION 1 ALONG WITH HEAVY METAL CONCENTRATIONS IN MUSSELS (X̄ CONCENTRATIONS)

| Ref. | Studies in | | Temp. (°C) | pH | D.O.* | $NO_3$* | $PO_4$* | Salinity o/oo | Pb* | Zn* | Fe* | Mn* | Cr* | Cd* | Cu* | As* | N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | JUNE, 1973 | A | 17.4 | 8.0 | 8.0 | 0.07 | 0.3 | 10.0 | - | - | - | - | - | - | - | - | 27 |
| | JULY, 1973 | A | 21.2 | 7.9 | 7.9 | 0.09 | 0.05 | 14.6 | - | - | - | - | - | - | - | - | 30 |
| | JUNE, 1973 | A | - | - | - | - | - | - | .16 | .07 | .85 | .34 | .02 | 2 | - | - | 10 |
| 2 | 1973 | A | - | - | - | - | - | - | .02 | .02 | - | - | - | 0.01 | 0.05 | - | - |
| 2 | 1974 | A | - | - | - | - | - | - | .05 | .01 | - | - | - | 0.01 | 0.04 | - | - |
| 2 | 1975 | A | - | - | - | - | - | - | .01 | 0.01 | - | - | - | < .01 | .02 | < .01 | - |
| 1 | JUNE, 1973 | A | - | - | - | - | - | - | .78 | 0.71 | 1.35 | .24 | .12 | .02 | - | - | 12 |
| 1 | JUNE, 1973 | A | - | - | - | - | - | - | .07 | .33 | .83 | 2.7 | < .01 | < .001 | - | - | 6 |
| 1 | JUNE, 1973 | A | - | - | - | - | - | - | .09 | .06 | .36 | .24 | .01 | < .001 | - | - | 12 |
| 3 | MAY, 1974 | A | 9.6 | 7.6 | 7.5 | - | - | - | .1-.3 | - | - | - | - | 0.02 | - | - | 18 |
| 3 | JUNE, 1974 | A | 13.4 | 7.6 | 8.0 | - | - | - | .1-.3 | - | - | - | - | - | - | - | 22 |
| 3 | JULY, 1974 | A | 19.5 | 7.6 | 8.0 | - | - | - | .1-.3 | - | - | - | - | - | - | - | 22 |
| 2 | 1972 | M | - | - | - | - | - | - | 107 | 49.7 | - | - | - | 3.4 | 3.7 | - | 3 |
| 2 | 1973 | M | - | - | - | - | - | - | 28.6 | 45.2 | - | - | - | 1.00 | 1.4 | - | 10 |
| 2 | 1974 | M | - | - | - | - | - | - | 190.1 | 59.5 | - | - | - | 6.5 | 8.4 | - | 3 |
| 2 | 1975 | M | - | - | - | - | - | - | 133.6 | 47.8 | - | - | - | 11.0 | 1.6 | < 1.0 | 24 |
| 4 | JULY, 1971 | A | 19.5 | 7.2 | 5.1 | - | - | 20.1 | - | - | - | - | - | - | - | - | 4 |
| 4 | JULY, 1972 | A | 14.1 | 7.8 | 9.0 | - | - | 3.7 | - | - | - | - | - | - | - | - | 4 |

A = Water; M = Mussels; *PPm; 1 = Basque et al, 1974; 2 = Dugdale et al, 1977; 3 = Blanchard et al, 1975;
4 = Bathurst Harbour; Ab = Brunswick Smelting; Ac = Nigadoo Mines; Ad = Consolidated Bathurst; N = Number of samples

TABLE 2 WATER QUALITY CHARACTERISTICS AND HEAVY METAL CONTENTS IN SURFACE WATERS (JUNE-SEPTEMBER 1973) OF REGION 2. ($\bar{X}$) CONCENTRATIONS IN P.P.M. A = IN WATER (N:45) B = IN OYSTERS (N=90)

| | | Mn | Ni | Cu | Zn | Hg | Pb | Ag | Fe | Salinity o/oo | Temp. | pH |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Caraquet | A | 0.21 | 0.21 | 0.09 | 0.07 | - | 0.19 | 0.01 | 0.35 | 20.8 | 19.5 | 8 |
| | B | 126 | 157 | 9.5 | 3.4 | 1.4 | - | - | - | - | - | - |
| St-Simon | A | 0.15 | 0.51 | 0.90 | 0.07 | - | 0.18 | 0.01 | 0.28 | 22.9 | 18.5 | 8 |
| | B | 137 | 139 | 10.2 | 8.2 | 0.9 | - | - | - | - | - | - |
| Shippagan | A | 0.18 | 0.44 | - | 0.07 | - | 0.18 | - | 0.20 | 24.4 | 19.9 | 8 |
| | B | 98.2 | 133 | 5.4 | 19.1 | 0.62 | - | - | - | - | - | - |
| Tracadie | A | 0.15 | 0.53 | 0.10 | 0.08 | - | 0.19 | 0.02 | 0.18 | 19.9 | 18.3 | 8 |
| | B | 104.8 | 132.2 | 4.06 | 13.6 | - | - | - | - | - | - | - |
| Tabusintac | A | 0.23 | 0.56 | 0.11 | 0.08 | - | 0.2 | 0.01 | 0.35 | 15.7 | 20.5 | 8 |
| | B | 107 | 125.4 | 4.8 | 7.5 | 0.67 | - | - | - | - | - | - |
| Neguac | A | 0.19 | 0.48 | 0.04 | 0.07 | - | 0.18 | 0.01 | 0.03 | 24.2 | 17.9 | 8 |
| | B | 79.2 | 146.5 | 9.4 | 10.2 | 0.76 | - | - | - | - | - | - |
| Sheldrake Is. | A | 0.13 | 0.48 | 0.06 | 0.07 | - | 0.19 | 0.01 | 0.03 | 18.3 | 17.6 | 8 |
| | B | 134 | 120.4 | 5.4 | 12.9 | - | - | - | - | - | - | - |
| Riv. Black | A | 0.15 | 0.42 | 0.10 | 0.09 | - | 0.17 | 0.02 | 0.47 | 16.0 | 20.5 | 8 |
| | B | 98.8 | 212 | 0.08 | 10.2 | .55 | - | - | - | - | - | - |
| Baie du Vin | A | 0.19 | 0.46 | 0.08 | 0.07 | - | 0.20 | 0.01 | 0.25 | 17.1 | 20.9 | 8 |
| | B | 92.6 | 215.5 | 5.97 | 11.06 | .94 | - | - | - | - | - | - |

TABLE 3 SURFACE WATER QUALITY OF CARAQUET BAY DURING JUNE TO NOVEMBER 1982
N = 48; ($\bar{X}$) CONCENTRATIONS - REGION 2

| Parameter | June | July | August | September | October | November |
|---|---|---|---|---|---|---|
| Temperature (°C) | 16.3 | 18.4 | 17.7 | 17.9 | 8.5 | 2.8 |
| Salinity o/oo | 28.7 | - | 26.3 | 27.8 | 26.6 | 27.8 |
| pH | 7.9 | - | 8.2 | 8.1 | 7.9 | 8.1 |
| Turbidity (PPM) | 9.6 | 3.3 | 5.7 | 1.5 | 1.5 | 3.8 |

TABLE 4 COASTAL WATER QUALITY OF REGION 3 $(\bar{X})$ CONCENTRATIONS IN P.P.M.

| Place of Study | | Studies in | No. of Samples | Water Temp. | D.O.* | Salinity o/oo | pH | Free $CO_2$ | Turbidity | $NO_3$* | $PO_4$* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Shediac Bay | May, 1972 | 90 | 9.8 | 9.0 | 7.9 | 7.6 | 5.6 | 5.2 | - | - |
| | - DO - | May, 1972 | 90 | 9.9 | 9.0 | 14.2 | 7.6 | 7.0 | 6.0 | - | - |
| | Bouctouche Bay | June, 1973 | 12 | 20.5 | 8.0 | 23.2 | - | - | - | - | - |
| | - DO - | July, 1973 | 27 | 21.1 | 7.5 | 20.3 | - | - | - | - | - |
| | - DO - | August, 1973 | 15 | - | 7.0 | 19.6 | - | - | - | - | - |
| | Northumberland Strait | 1975 (June-August) | 95 | 17 | - | 23.7 | - | - | - | - | - |
| 2 | - DO - | 1976 (May-October) | 185 | 13.4 | 6.2 | 24.6 | 7.9 | - | - | .02 | .03 |
| 2 | - DO - | 1977 (May-October) | 185 | 11.6 | 6.9 | 25.7 | 8.1 | - | - | .06 | .007 |

1 = Lakshminarayana and Jean-Pierre (1975);
2 = Lakshminarayana and Bourque (1979);
* P.P.M.

TABLE 5 CONCENTRATION OF HEAVY METALS IN THE COASTAL WATERS OF NORTHUMBERLAND STRAIT (mean values in µ g/L)

| | REGION 3 | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1976 | | | | | | 1977 | | | | | |
| | X̄ | | Max | | Min | | X | | Max | | Min | |
| Metal | S | B | S | B | S | B | S | B | S | B | S | B |
| Fe | 2.8 | 2.5 | 6.0 | 3.8 | 1.8 | 1.2 | 350 | 43 | 600 | - | 300 | - |
| Mn | 1.8 | 1.9 | 5.0 | 5.0 | 0.8 | 0.6 | 70 | - | 80 | - | 60 | - |
| Cu | 0.8 | 1.1 | 1.4 | 2.3 | 0.4 | 0.4 | 17 | - | 50 | - | 4 | - |
| Ni | 14.6 | 14.9 | 20 | 20 | 10 | 10 | - | - | - | - | - | - |
| Zn | 0.9 | 0.8 | 2.8 | 1.8 | 0.3 | 0.4 | 4 | - | 50 | - | 2 | - |
| Pb | 3.5 | 4.0 | 6.0 | 6.4 | 2.5 | 2.5 | 2.0 | - | 6.0 | - | 2.5 | - |
| Cd | 0.6 | 0.7 | 0.8 | 1.0 | 0.5 | 0.6 | 1.0 | - | 3.0 | - | 0.6 | - |

S = surface; B = bottom; X̄= mean; Max = maximum; Min = minimum; - not estimated.
N = Number of samples

TABLE 6 WATER QUALITY CHARACTERISTICS OF MALPEQUE BAY, P.E.I. - REGION 4

*P.P.M.

| | Malpeque Bay - Present Study | | | | | |
|---|---|---|---|---|---|---|
| | (N1 to N6 Stations (June, 1973-July, 1974) | | | M1 to M16 Stations (Summer, 1974) | | |
| | Maximum | Minimum | Average | Maximum | Minimum | Average |
| W.T. (°C) | 26.0 | -1.4 | 17.4 - 9.2 | 24.8 | 19.4 | 21.9 |
| pH | 8.9 | 7.0 | 8.2 - 8.0 | 9.2 | 7.4 | 8.2 |
| Transparency | - | - | - | 3.7 | 1.1 | 2.1 |
| Salinity o/oo | 27.9 | 0.1 | 25.9 - 23.3 | 32.6 | 27.4 | 29.7 |
| D.O.* | 10.6 | 0.7 | 6.5 5.5 | 6.9 | 3.2 | 4.7 |
| $NO_3$-N* | 0.51 | 0.03 | .26 .14 | 0.076 | .002 | .002 |
| $NO_2$-N* | 0.041 | 0.001 | .015 - .008 | .004 | .001 | .002 |
| $NH_3$-N* | 0.09 | 0.002 | .048 - .018 | .018 | .005 | .011 |
| Kj-N* | 0.79 | 0.14 | 0.54 - 0.32 | .31 | .11 | .21 |
| $PO_4$-P* | 0.06 | 0.002 | 0.039 - 0.021 | .007 | .001 | .003 |
| $PO_4$-T&P* | 0.084 | 0.009 | 0.065 - 0.034 | .013 | .004 | .009 |
| Si* | 0.38 | 0.01 | 0.13 - 0.09 | .034 | .008 | .014 |
| Zn* | 0.06 | 0.01 | - | - | - | - |
| Cu* | 0.04 | 0.01 | - | - | - | - |
| Fe* | 0.04 | 0.01 | - | - | - | - |
| Ni* | 0.06 | 0.01 | - | - | - | - |
| Pb* | 0.03 | 0.01 | - | - | - | - |
| Mn* | 0.03 | 0.01 | - | - | - | - |
| Cd* | 0.006 | 0.001 | - | - | - | - |
| Hg* | 0.006 | 0.001 | - | - | - | - |

catch value increased despite the drop in catch volume. Is this situation partly due to pollution of the coastal waters? The coastal water is of acceptable quality (Table 1). Temperature and salinity fluctuated depending on the weather and particularly the latter on the autochthonic and allochthonic inputs. Surface values for phosphate-P usually fall within 0-20 μg/L in coastal waters (Tait an DeSanto, 1972). The orthophosphate concentrations were more than 0.3 ppm in several places indicating probable eutrophication. In general, the heavy metal contents were below tolerance limits of marine waters which are: Pb (0.05), Zn (20), Mn (0.1), Cr (0.05), Cd (0.01), Cu (1.5-3.0), As (0.2) ppm. However coastal waters (Dugdale, 1977) near Belledune Fertilizer, Brunswick Smelter and Consolidated Paper and Pulp, the metal contents of the waters were elevated although the variations were extreme. The average tolerance values for Pb and Cd were near or above the tolerance limits for marine waters (Table 1). The pH, at some of these areas, was less than 5. Regions of Caraquet, Bas-Caraquet, Youghall and Petit Rocher had coastal waters with very high concentrations of coliforms (Basque et al, 1974). Dugdale et al (1977) also showed similar increases in the Belledune Smelter and Harbour area and they recorded elevated concentrations of heavy metals in mussels (Table 1) and other aquatic organisms. Loring et al (1980) stated that Zn and Cd were in considerably increased levels in water (260 μg/L) while copper concentrations were comparable to the other coastal waters. High cadmium levels in sediments and lobsters from Belledune harbour were attributed to air and water emissions by Matheson and Baker (1980). They surmised that Cd contamination of Dalhousie harbour was due to leaching of stored ore concentrate and thermal power generation station. Varied concentrations of Cd were detected in digestive glands, hepatopancreas (20 $\mu g/g^{-L}$-Zitko, 1981) and tail muscles of lobster (Uthe, Chou and Robinson, 1980; and Uthe and Freeman, 1980). In the marine biota Ray et al (1980) also recorded Cd in the vicinity of Belledune. The above studies indicated positive accumulation of heavy metals by the coastal fauna and flora.

Region 2 showed good coastal surface water quality (Tables 2 and 3). The heavy metals (Table 2) in the waters were within the tolerance limits of the marine environment. The oysters showed accumulations of heavy metals. The pH of these coastal waters is higher than those of the regions 1 and 3.

Waters of Northumberland Strait (Region 3) are subjected to pollution from fresh water, natural drainage, sewage and other industrial wastes of local communities in the southern and northern regions. For example, Culligan and Baster (1973) and Lakshminarayana and Jean-Pierre (1975) reported bacterial contamination. The shore regions are subjected to tidal current velocities of 0.5 knots and higher, and the estuaries trap most of the sediment load (Farquharson 1962; Kranck 1971). The transparency of the waters varied and the waters were well oxygenated (Table 4). Bacon (1977) reported dissolved oxygen values above 5 ppm in Northumberland Strait and that the waters were isohaline during December following ice formation. However, thermal stratification was observed at some stations only during November, April, May and June. Studies showed distinct differences in water temperatures and salinities of St. Edouard de Kent and Cap Pelé, the latter with higher values (Lakshminarayana and Bourque, 1979). The effect of spring thaw and run off was evident during March and April when the salinity were the lowest and the phosphorus was detectable (Bacon, 1977). Lakshminarayana and Bourque (1979) indicated a probable nutrient enrichment in the waters.

The concentration levels and distribution of iron, manganese, copper, nickel, zinc, lead and cadmium in Northumberland Strait waters are shown in Table 5. No unusual concentration of cadmium occurred. Copper, zinc, lead, nickel, manganese and iron showed levels characteristic of coastal waters. Although copper (0.8 μg/L) showed the same level as zinc (0.9 µg/L) during 1976, in 1977 the samples showed high levels (17.0 µg/L). Iron (300-600 µg/L), manganese (60-80 µg/L), and lead (2.0-6.0 µg/L) showed unusually high concentrations in 1977. This may be due to contribution of land run off as indicated by low salinity and pH. Also all samples show total iron as they were not filtered.

The water quality of Malpeque Bay (Table 6) favourably compares with the findings of Bartlet (1971) and McIver (1972) and Uyeno (1966) except for the dissolved nutrient fractions. The waters were alkaline and well oxygenated. The nitrate nitrogen and orthophosphate showed a ratio of 7:1. At N stations trace metal concentrations in surface and bottom waters were mostly found to be in similar ranges. Variations in trace metals at all the N stations are as follows:

Zn & Ni - 0.01 to 0.06
Cu, Fe, - 0.01 to 0.04
Pb & Mn
Cd - 0.001 to 0.006
Hg - 0.001 to 0.003

Seasonal variations in trace metal concentrations were not distinguishable but differences in distribution of trace metals at various sampling stations existed.

Manganese and lead showed a maximum concentration of 0.03 ppm and copper and iron a concentration of 0.04 ppm at N1. Maximum concentration of 0.006 ppm for cadmium was recorded at N1 station. Zinc, nickel, manganese and lead varied from 0.01 and 0.03 ppm at N2. Cadmium showed a range of 0.001 to 0.003 ppm at N2 and N4 and N6. Nickel and lead varied between 0.01 and 0.02 ppm in the waters of N4 station. Zinc showed higher concentrations of 0.06 ppm.

At N5 cadmium registered a maximum concentration of 0.004 ppm while zinc, iron, and nickel ranged from 0.01 - 0.04 ppm. Copper, lead and manganese showed a concentration of 0.02 ppm. Station N6 has shown a maximum concentration of copper, iron, lead and manganese as 0.03 ppm. Mercury showed a maximum concentration of 0.03 ppm at station N3. The heavy metal concentration of Malpeque Bay are comparable with these of ocean waters (Zn = 0.01; Fe = 0.01; Ni = 0.002; Cu = 0.003; Cd = 0.0001; Pb = 0.00003; Fe = 0.01; Mn = 0.002 ppm).

Philips (1977) suggested that the macroalgae and bivalve molluscs are the most efficient and reliable indicators to monitor trace metal pollution in marine and estuarine environments. Philpott (1978) found from his literature survey that oysters and soft shell quahaug clams have rapid rates of uptake and high tissue concentrations of heavy metals. Concentrations of 1000 ppm of zinc and 30 ppm copper were reported in drained oyster meats. Goldberg (1975, 1978) proposed the "mussel watch" to record how man's activities are altering oceanic composition. Cadmium concentrations in the blue mussels (*Mytilus edulis*) around Cape Breton, N.S., were reported to contain maximum concentrations of 2.34 µg/g$^{-1}$ dry weight and this concentration was found to be lower than those in mussels from industrialized estuaries in Baltic or Northeast coast of U.S. (Mobile Oil Canada Ltd., 1983). Exposure of 5.0 ppb Cd for 40 weeks resulted in elevated Cd levels (13.6 ppm - wet weight) in the tissues (Zaroogian and Cheer, 1976). Oysters (*Crassostrea virginica*) exposed to 15 ppb cd in water for 40 weeks, followed by a 16 week depuration period did not change the accumulated cadmium levels (Zitko, 1980). Clams and oysters accumulated equally well Fe, Zn and Cu in the chocolate and Jones Bays of the Gulf Coasts of

Texas although Barnacles and polychaetes were found to have highest concentrations of many heavy metals (Guthrie et al, 1979). Oysters growing nearest urban areas were found to have highest concentrations of one or more metals (Ratkowsky et al, 1974). The coastal waters of New Brunswick and P.E.I. were found to be of good quality in regions where urbanization and industrialization has not yet influenced. With the present stringent measures against pollution by the regulating agencies we can hope for improvement in future and thus may be conducive for establishing extensive mariculture operations such as expanding or re-establishing oyster hatcheries and clam farms.

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# BLUE MUSSEL CULTURES AS A BIOMONITORING TOOL FOR LEAD, ZINC AND CADMIUM CONTAMINATION

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PRAIRIE, R. and R.L. LEVAQUE CHARRON. 1985. Blue mussel cultures as a biomonitoring tool for lead, zinc and cadmium contamination. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 199-220.

Blue mussels (Mytilus edulis) were utilized to monitor spatial and temporal distribution of Pb, Zn and Cd in the marine environment in the vicinity of a lead smelter. Mussel specimens (size range 20-30 mm total length) were collected from a reference area and relocated at various sites bracketing the effluent outfall area.

Each station consisted of a wooden support rack attached to a buoy-anchor system on which nine mesh bags each containing over fifty mussels were hung in order to allow free passage of water through the bags. At approximately 25-day intervals over a three-month period, three bags were collected from each station for chemical analysis of homogenized soft tissues.

This methodology allowed observation of the bioaccumulation rate at each location and overall spatial and temporal bioaccumulation patterns for the area during the study period. From these results, contamination zones were delineated and overall dispersion patterns of contaminants derived.

This presentation describes the methodology, and overall results are included to illustrate the trends obtained.

PRAIRIE, R. and R.L. LEVAQUE CHARRON. 1985. Blue mussel cultures as a biomonitoring tool for lead, zinc and cadmium contamination. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 199-220.

La moule bleue (Mytilus edulis) a été utilisée pour surveiller la distribution spatiale et temporelle du plomb, du zinc et du cadmium dans l'environnement marin à proximité d'une fonderie de plomb. Des spécimens de moules (d'une longueur totale comprise entre 20 et 30 mm) ont été rassemblés à partir d'une aire de référence et redistribués à différents endroits encadrant la zone de décharge de l'effluent.

Chaque station était constituée d'un cadre de bois attaché à un système de bouée sur lequel étaient accrochés neuf poches en filet maillant contenant chacun plus de cinquante moules, de façon à permettre le libre passage de l'eau à travers les poches. A intervalles

d'environ 25 jours, sur une période de trois mois, trois sacs ont été recueillis à chaque station, en vue de faire une analyse chimique des tissus mous homogénéisés.

Cette méthode a permis d'observer le taux de bioaccumulation à chaque endroit, ainsi que les schémas de bioaccumulation dans l'espace et dans le temps pour la région au cours de la période d'étude. Ces résultats ont permis de délimiter des zones de contamination et de déterminer des schémas de dispersion.

Dans notre communication, on trouvera l'exposé de la méthode suivie et des résultats indiquant les tendances observées.

## INTRODUCTION

In order to assess the effects of industrial operations and activities on the environment, scientist have often used bioindicators to monitor the presence and behavior of contaminants(1,2). By accumulating contaminants within their tissues these bioindicators provide a quantitative estimate of the relative degree of contamination within their environment.

Qualifications of a bioindicator organism include (Figure 1):

- ability to accumulate contaminants in its tissues without being killed;
- abundance and ease of collection;
- indegenous and also sedentary in nature.

Molluscs, particularly bivalves such as clams, oysters and mussels, have long been recognized as prime bioindicators. In temperate waters, a suitable candidate is the blue mussel, *Mytilus edulis* (Figure 2).

This presentation (Figure 3) briefly describes the background of blue mussel studies and the types of studies that can be carried. A case study is then presented in detail, with emphasis on the methodology and the treatment of the results.

### Background of Blue Mussel Studies

Numerous mussel studies have been carried out at Belledune, on the northeastern coast of New Brunswick, where Brunswick Mining and Smelting Corporation Limited has operated a primary lead smelter since 1968. The smelting and refining operations entail the use and contamination by heavy metals of a certain volume of freshwater. Part of that water is recycled in the process and the rest is discharged in the Baie des Chaleurs as an effluent containing heavy metals such as Cd, Pb and Zn. In 1980, treatment of the effluent was initiated.

Since 1972, Noranda Research and Brunswick Smelting has collaborated in monitoring programs to assess the degree and extent of heavy metal contamination in the vicinity of the industrial area, including using blue mussels.

### Types of Studies

The mussel studies can be primarily divided into two types, using the concept of bioaccumulation on a *long* and *short* term basis (Figure 4).

The evaluation of *long term* bioaccumulation of contaminants by mussels (called **MUSSEL COLONY STUDY**) is done by sampling native mussels at various distances from the source(s) of contamination.

# HOW TO QUALIFY AS A BIOINDICATOR

- able to accumulate contaminants without being killed
- abundant and easy to sample
- indigenous and sedentary

FIGURE 1 HOW TO QUALIFY AS A BIOINDICATOR

# BLUE MUSSEL AS A BIOINDICATOR

- sessile (sedentary)
- shoreline environment
- clusters
- filter feeders

FIGURE 2 BLUE MUSSEL AS A BIOINDICATOR

# OUTLINE

**Background of blue mussel studies**

**Types of studies**

**Case study (flowchart)**

- methodology
- treatment of results

FIGURE 3 OUTLINE

# TYPES OF STUDIES

| CULTURES | COLONIES |
|---|---|
| relocation | on site (native) |
| short term | long term |
| labor intensive | simple collection |
| in and out of intertidal zone ( three dimensional) | intertidal zone only (uni-dimensional) |

FIGURE 4 TYPES OF STUDIES

The assessment of short term bioaccumulation by mussels **(MUSSEL CULTURE STUDY)** consists of relocating uncontaminated specimens to different locations near potential contamination sources. In addition to the difference in exposure period, cultures can be relocated in and out of the intertidal zone (three dimensional), whereas colonies are found mainly in intertidal zone (uni-dimensional shoreline). However, a culture study requires more intensive labor to relocate and house the organisms than the simple collection of native mussels along the coast.

Figure 5 is based on a mussel colony study and shows the variation of Cd levels in native mussels collected in 1980(3), in the intertidal zone, on both sides of Belledune Harbour. The results permit:

- identification of the main sources of contamination;
- determination of the degree and extent of Cd impact;
- definition of zones of different Cd levels of impact;
- illustration of overall trends (inter-year comparison).

### Case Study - 1981 Mussel Culture(4)

Figure 6 is a flowchart of the case study showing the different steps involved for such a program.

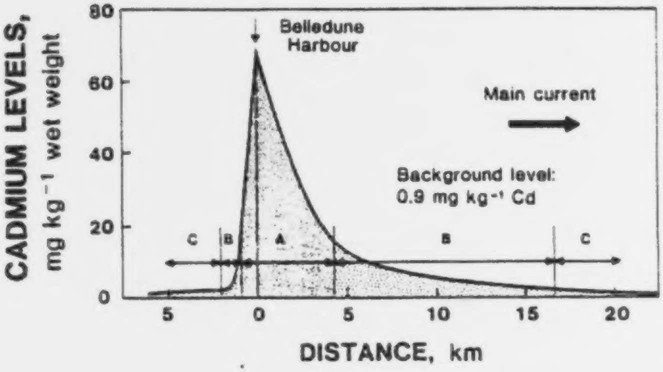
First, what was the reason for this study? (Figure 7.)

A mussel culture study was carried out in the summer of 1980 at strategic locations within Belledune Harbour. In November 1980, the treatment of the waste waters from the smelters operations was initiated in order to reduce the concentration of cadmium and other contaminants in the effluent; the outfall was also relocated outside Belledune Harbour.

The objective of the 1981 study was to: 1) define the zones of different levels of heavy metal bioaccumulation by mussels, and approximate contaminants dispersion patterns and 2) assess the effects of both the treatment of waste waters and the relocation of the main outfall on bioaccumulation, using the 1980 results for comparison.

The collection of mussels clusters was done by snorkeling in the intertidal zone of an uncontaminated area. Debris such as empty shells or other marine species were removed. In the first years that such studies were carried out, no distinction was made regarding the size of mussels collected. However, based on the literature(5-9), a smaller size range group was sampled in 1981 (i.e., 20 to 30 mm length mussels) since bigger specimens may have seasonal variation of their metal tissue content caused by physiological changes such as sexual maturity. Mussels were then pooled in groups of fifty specimens per sample to reduce individual variations and then three samples per station (per sampling date) were prepared in order to evaluate sample variation.

# MUSSEL COLONIES



- identify or confirm contaminant source
- evaluate degree and extent of impact
- define zones of impact (A, B, C)
- overall trend identification

FIGURE 5 MUSSEL COLONIES

**CASE STUDY FLOWCHART**

1. Background
2. Objectives
3. Fieldwork
    - collection
    - sorting
    - installation
    - exposure
    - retrieval
4. Laboratory work
    - preparation
    - analysis
5. Assessment of results
6. Conclusions

FIGURE 6 CASE STUDY FLOWCHART

# CASE STUDY
## 1981 MUSSEL CULTURE PROGRAM

**Background**

- 1980 bioaccumulation study
- November 1980
    - water treatment
    - relocation of outfall

**Objectives**

1. Define bioaccumulation zones and dispersion patterns
2. Assess effects of water treatment plant on bioaccumulation

FIGURE 7 CASE STUDY - 1981 MUSSEL CULTURE PROGRAM

Each group of 50 mussels was then placed into Vexar envelopes (900 cm$^2$). Once transferred to the study area, these bags were set up in rows of three (one rope per sampling date) on a clothes line arrangement within a wooden frame (Figure 8). This frame was held at one to two meters below the surface by means of a buoy-anchor system.

(It should be noted that the technique was further simplified for 1982 and 1983 surveys by not using the wooden rack; instead, the mussel envelopes were attached directly to the anchor-buoy line.)

A total of 18 stations were installed at various locations in the vicinity of the two outfalls (Figure 9) for a period of approximately 85 days during which time three samplings were carried out (at 25-30 day intervals). The location of some of these stations were established by preliminary dye tracer test, underwater observations and salinity readings near the new outfall.

During the main survey period, the conditions of stations and mussels were checked routinely, and repairs executed if necessary.

After retrieval, the samples were brought to the laboratory for preparation. The mussels were shucked and homogenized using a regular type kitchen blender and/or a polytron tissue homogenizer. Tissue was then kept in sterile whirl-paks prior to analysis (about 20 g).

The Pb, Zn and Cd determination were carried out by the Environmental Laboratory of Brunswick Smelting Division, using flame atomic absorption spectroscopy method. The analytical results were checked using reference materials and replicates, and also via cross-checks with one other laboratory.

We had, therefore, Pb, Zn and Cd results for 18 different locations and three different dates (Figure 10). These results were grouped in two different ways before any treatment was done. First, the variations in the time were assessed using fluctuations observed at each station throughout the study period. The second way was to assess variations in location (spatial variations) using levels observed at all stations for each sampling date. These two different groupings, respectively, provided bioaccumulation curves and patterns from which zones of contamination were derived. Finally, dispersion patterns for the study area were approximated.

Figure 11 shows the first way of grouping these results, illustrated by graphs for one "high" and one "low". Statistical analysis was also carried out, using the student "t" test, on each group of data, in order to determine the significance of increases or decreases observed. From these graphs, it is fairly easy to determine the highest and lowest bioaccumulation rate but is harder to detect the overall trend in relation with station location.

The second grouping method (all stations results grouped for each sampling date) examined the significance of differences between all stations for one sampling date, statistically, by means of an analysis of variance (ANOVA), combined with a Student Newman Keuls test(10). A non-parametric test, the similarity analysis(11), was also utilized. This was the Gower coefficient along with a clustering method (unweighted pair grouped method), which grouped stations with similar results. This method has often been used in benthos or plankton studies but also in studies involving chemical results.

## MUSSEL CULTURE SET-UP

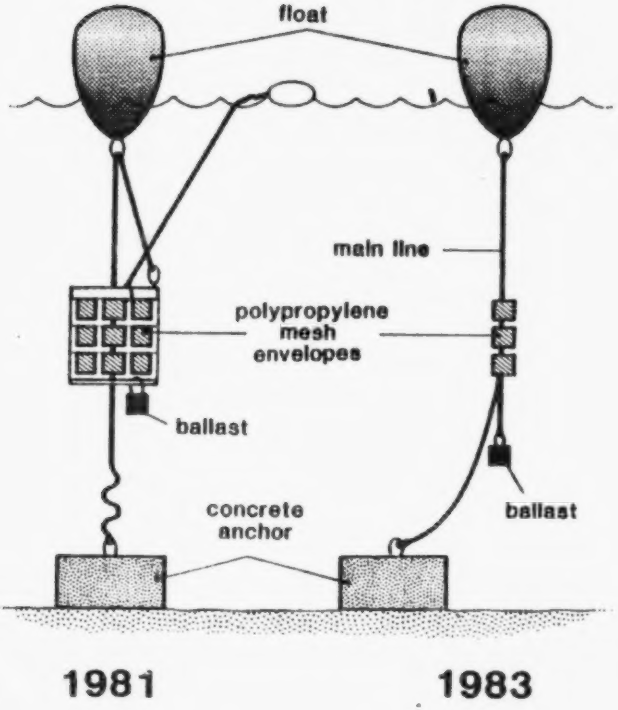
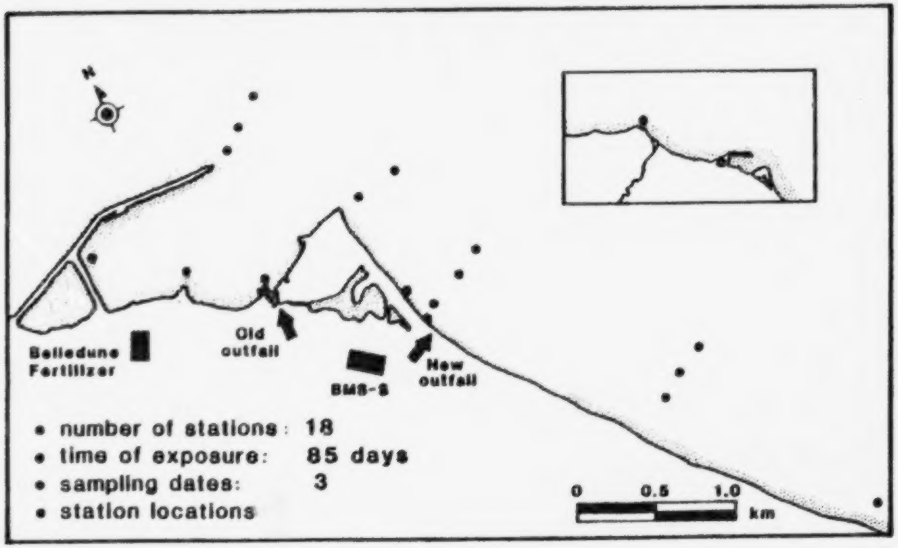

float
main line
polypropylene mesh envelopes
ballast
concrete anchor
ballast
1981
1983

FIGURE 8 MUSSEL CULTURE SET-UP

## MUSSEL CULTURE STATION LOCATIONS


N
Belledune Fertilizer
Old outfall
BMS-S
New outfall
0
0.5
1.0
km

- number of stations: 18
- time of exposure: 85 days
- sampling dates: 3
- station locations

FIGURE 9 MUSSEL CULTURE STATION LOCATIONS

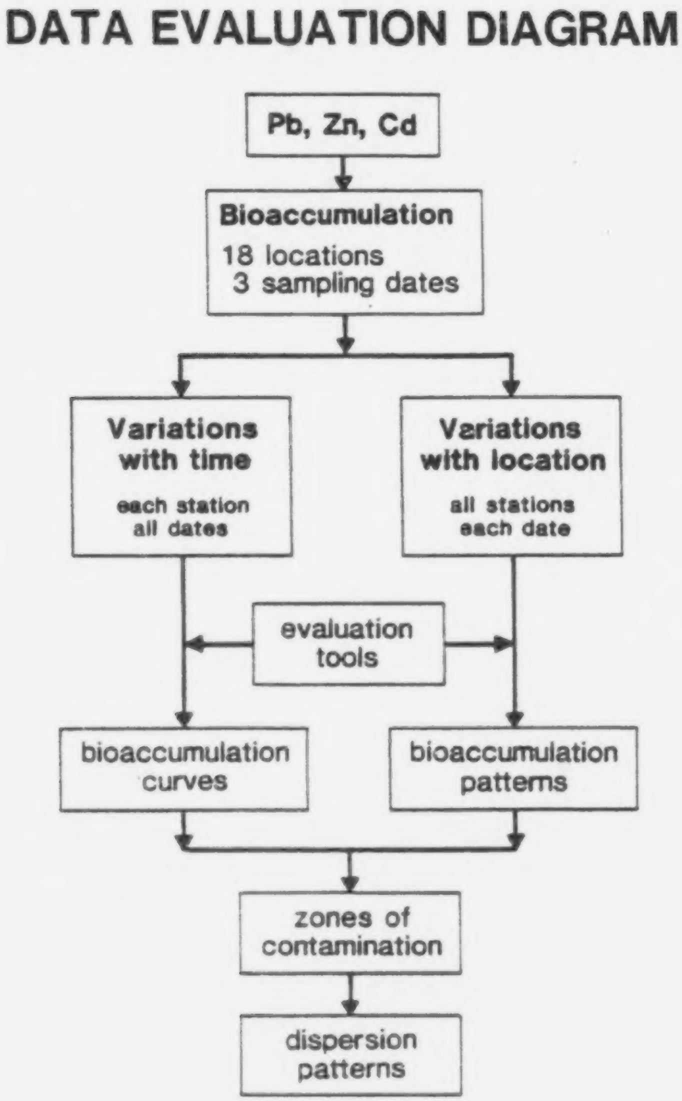

DATA EVALUATION DIAGRAM
Pb, Zn, Cd
Bioaccumulation
18 locations
3 sampling dates
Variations with time
each station
all dates
Variations with location
all stations
each date
evaluation tools
bioaccumulation curves
bioaccumulation patterns
zones of contamination
dispersion patterns

FIGURE 10 DATA EVALUATION DIAGRAM

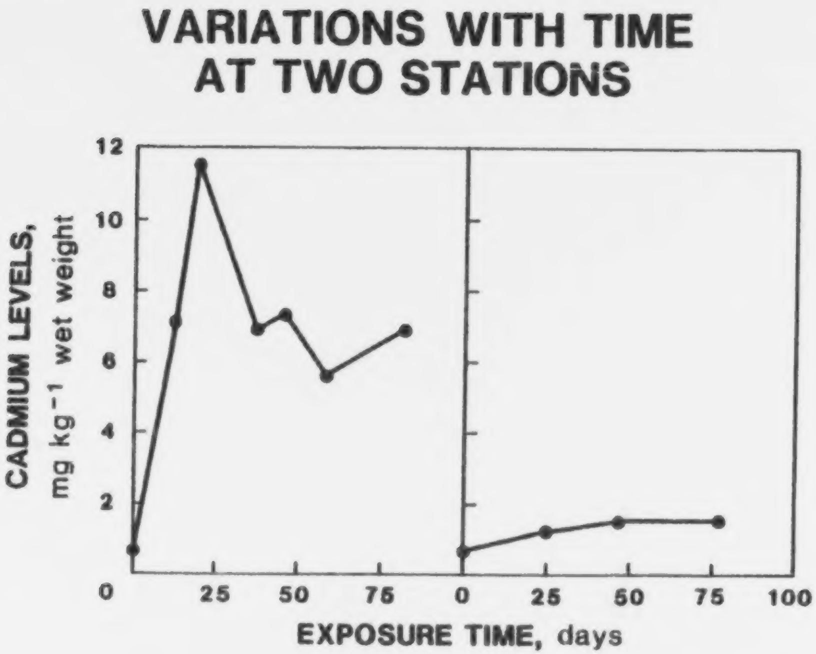


FIGURE 11 VARIATIONS WITH TIME AT TWO STATIONS

The grouping of similar stations results can be illustrated on the survey map for each sampling date (Figure 12). For the three sampling dates, an integration (not a mean) of Pb, Zn and Cd results are plotted. This representation of the data shows the spatial and temporal variations of Pb, Zn and Cd at intervals during the study period.

Finally, the interpretation of these bioaccumulation patterns and the bioaccumulation curves (previously described) allowed (Figure 13):

- determination (or confirmation) sources of contamination;
- classification of zones of bioaccumulation (highly, moderately and slightly contaminated);
- approximation of the contaminant dispersion patterns.

The 1981 results were then compared with the pre-treatment plant results in 1980 (Figure 14). This figure shows the Cd levels observed in cultured mussels installed at three different locations in Belledune Harbour. A significant decrease of cadmium uptake by mussels was observed which can be related to the relocation of the main outfall outside the harbour.

## SUMMATION (Figure 15)

This presentation has outlined the concept of using mussels as bioindicators of Pb, Zn and Cd contamination. Mussel cultures have been compared briefly to mussel colonies and their differences noted. To illustrate the culture study technique, the 1981 Mussel Culture Program carried out for Brunswick Smelting Division was described, with emphasis on methodology and treatment of results. The results confirmed sources of contamination, illustrated spatial and temporal variations of some heavy metals, defined zones of bioaccumulation, approximate contaminants dispersion patterns. Finally, using inter-year comparisons, the results indicated a significant decrease of heavy metal bioaccumulation by cultured blue mussels in the vicinity of Brunswick Smelting Division operations, subsequent to start-up of a treatment plant in November 1980. This trend was also confirmed by colony samples from the harbour area.

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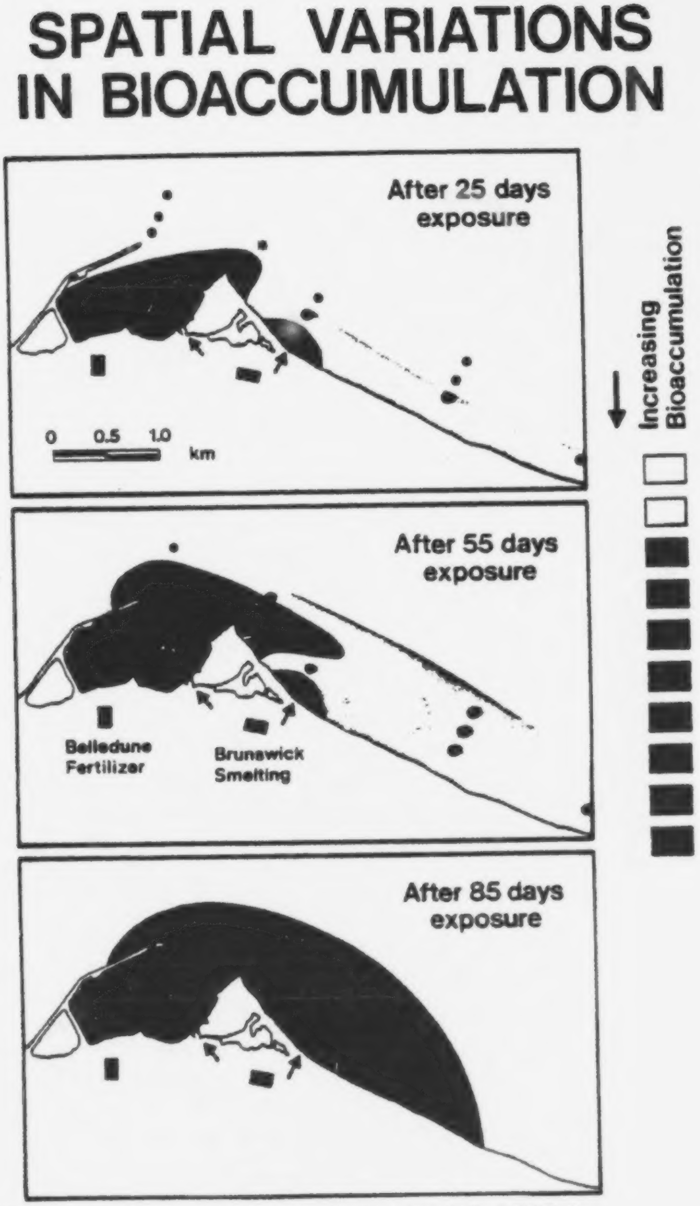
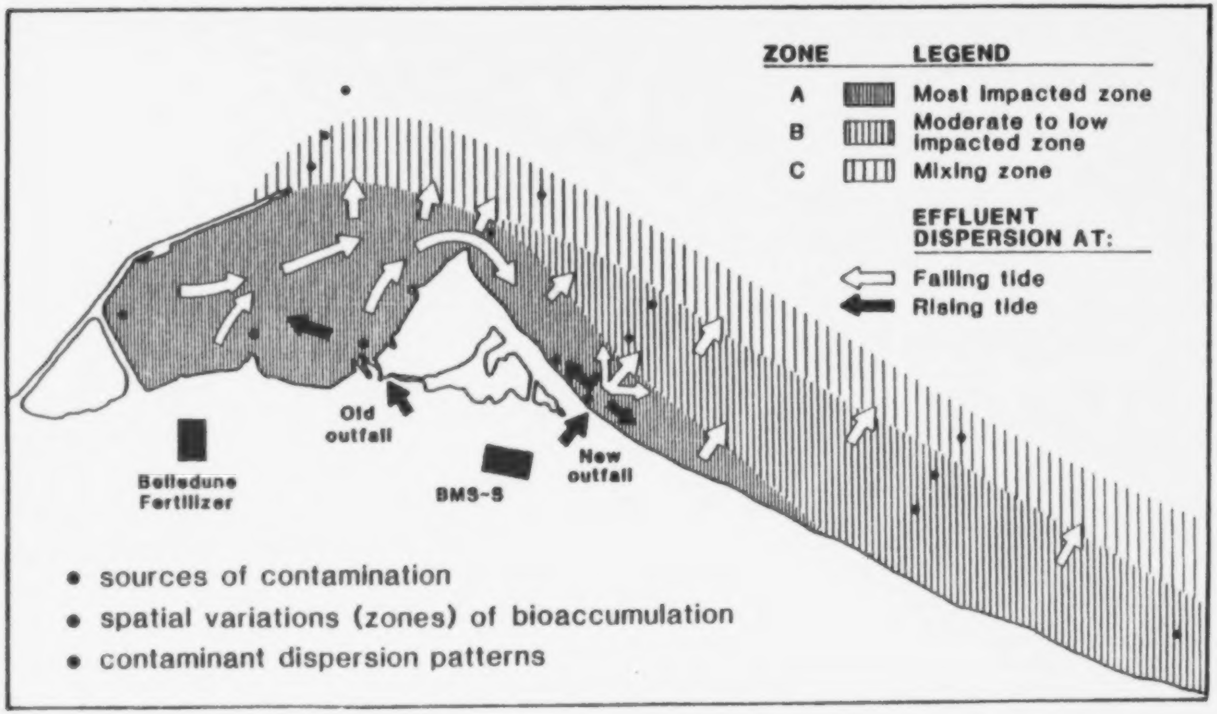

SPATIAL VARIATIONS IN BIOACCUMULATION
After 25 days exposure
0
0.5
1.0
km
After 55 days exposure
Belledune Fertilizer
Brunswick Smelting
After 85 days exposure
Increasing Bioaccumulation

FIGURE 12 SPATIAL VARIATIONS IN BIOACCUMULATION

# BIOACCUMULATION ZONES AND DISPERSION PATTERNS


ZONE
LEGEND
A Most impacted zone
B Moderate to low impacted zone
C Mixing zone
EFFLUENT DISPERSION AT:
Falling tide
Rising tide
Old outfall
New outfall
Belledune Fertilizer
BMS-S

- sources of contamination
- spatial variations (zones) of bioaccumulation
- contaminant dispersion patterns

FIGURE 13 BIOACCUMULATION ZONES AND DISPERSION PATTERNS

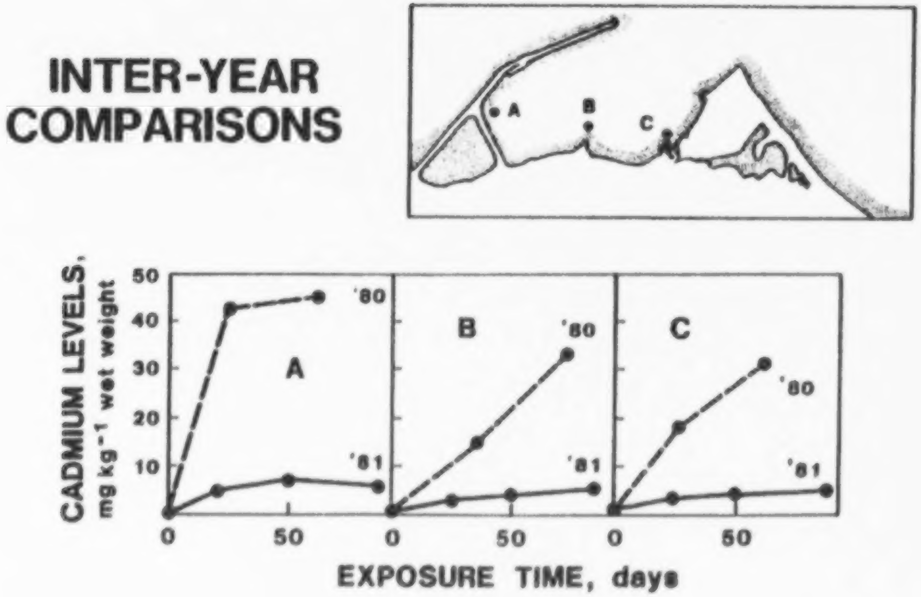


FIGURE 14 INTER-YEAR COMPARISONS

# SUMMARY

**Mussels as bioindicators**

**Colonies and cultures**

**Case study (culture)**

- sources of contamination
- spatial and temporal variations
- zones of bioaccumulation
- dispersion patterns
- inter-year comparisons

FIGURE 15 SUMMARY

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# DETERMINATION OF DIALKYL-, TRIALKYL, TETRAALKYLLEAD AND LEAD (II) COMPOUNDS IN WATER, SEDIMENT, FISH AND AQUATIC WEEDS.

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CHAU, Y.K. and P.T.S. WONG. 1985. Determination of dialkyl-, trialkyl, tetraalkyllead and lead (II) compounds in water, sediment, fish and aquatic weeds. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 221-222.

Determination of dialkyl- and trialkyllead compounds in biological samples has historically been difficult because of (1) their highly polar property which precludes quantitative extraction from sample matrices, and (2) their thermal instability which causes decomposition during chromatographic separations. Other techniques including polarography and spectrophotometry suffer either from lack of sensitivity or non-specificity with regard to the identity of the alkyl groups or the degree of alkyl substitution.

The present method based on quantitative chelation/extraction with diethyldithiocarbamate of the dialkyl- and trialkyllead from environmental samples and subsequent butylation with Grignard reagent of these compounds to the tetraalkyl substituted forms, $R_nPbBu_{(4-n)}$, and Pb (II) to $Bu_4Pb$, all of which can be quantified by the gas chromatography-atomic absorption spectrometry method. Tetraalkyllead compounds $R_4Pb$, and mixed tetraalkyllead, $R_nR'_{(4-n)}Pb$, (R=Me,Et) are co-extracted by this procedure and are included in the determination. This method determines nine species of alkyllead and lead (II) directly and simultaneously without calculation by difference. It has been applied to the analysis of water, sediment, fish and macrophytes with detection limits of 10 ng/L, 13 ng/g, 8 ng/g and 8 ng/g respectively.

CHAU, Y.K. and P.T.S. WONG. 1985. Determination of dialkyl-, trialkyl, tetraalkyllead and lead (II) compounds in water, sediment, fish and aquatic weeds. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 221-222.

La détermination des composés de plomb dialkyle et trialkyle dans les échantillons biologiques a été difficile, pour les raisons suivantes: 1) leurs propriétés hautement polaires, qui s'opposent à leur extraction quantitative à partir de matrices d'échantillon et 2) leur instabilité thermique, qui provoque la décomposition au cours des séparations chromatographiques. D'autres techniques, comme la polarographie et la spectrophotométrie, ne sont pas assez sensibles, ou n'indiquent pas assez spécifiquement l'identité des groupes d'alkyles ou le degré de substitution alkyle.

La présente méthode est fondée sur la chélation/extraction quantitative avec le diéthyldithiocarbamate du plomb dialkyle et trialkyle provenant des échantillons environnementaux, suivie d'une butylation avec le réactif de Grignard conduisant aux formes

substituées de tétraalkyle, $R_nPbBu_{(4-n)}$, et Pb(II) jusqu'à $Bu_4Pb$, qui peuvent toutes être quantifiées au moyen de la méthode de chromatographie en phase gazeuse et de spectrométrie par absorption atomique. Les composés de plomb tétraalkyle $R_4Pb$ et le mélange de plomb tétraalkyle $R_nR'_{(4-n)}Pb$, (R=Me, Et) sont coextraits au moyen de cette méthode et sont inclus dans la détermination. Cette méthode permet de déterminer neuf types de plomb alkyle et de plomb(II) directement et simultanément, sans calcul par différence. Elle a été appliquée à l'analyse de l'eau, des sédiments, du poisson et des macrophytes avec des limites de détection de 10 ng/L, 13 ng/g, 8 ng/g et 8 ng/g, respectivement.

# THE OCCURRENCE OF ALKYLLEAD COMPOUNDS IN THE AQUATIC ENVIRONMENT.

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HODSON, P.V., P.T.S. WONG, Y.K. CHAU, B.R. BLUNT, O. KRAMAR, and D.M. WHITTLE. 1985. The occurrence of alkyllead compounds in the aquatic environment. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 223-224.

Alkyllead compounds are widespread in the aquatic environment but generally occur at low concentrations. However, a survey in 1981 of fish from the St. Lawrence River indicated very high lead contamination near a point source. Concentrations of total lead in the blood of carp, suckers and pike ranged from .010 to 57 mg/L. Eleven separate alkyllead compounds were identified in the carcass of these fish with concentrations of individual forms ranging from non-detectable to 68.6 mg/kg. The sum of all alkyllead compounds in carcass ranged from .024 to 144.5 mg/kg. There were strong correlations between total blood lead, total lead in carcass and the sum of alkyllead compounds in carcass. Contamination was detected in fish 14 km downstream of the source and in a few fish 16 km upstream, perhaps due to migration.

Surveys in 1982 confirmed the high levels of lead contamination. Carp contained the most alkyllead, followed by white sucker, northern pike, yellow perch and spottail shiners. Rock bass, brown bullheads and smallmouth bass were among the least contaminated species. High levels of lead were also found in sediment and macrophytes but not in clams, perhaps due to a discrete "plume" in the river.

HODSON, P.V., P.T.S. WONG, Y.K. CHAU, B.R. BLUNT, O. KRAMAR, and D.M. WHITTLE. 1985. The occurrence of alkyllead compounds in the aquatic environment. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 223-224.

Les composés de plomb alkyle sont répandus dans l'environnement aquatique, mais se trouvent généralement à de faibles concentrations. Cependant, en 1981, un relevé de poissons du Saint-Laurent a indiqué une contamination par le plomb très élevée à proximité d'une source ponctuelle. Les concentrations de plomb total dans le sang des carpes, des meuniers et des brochets variaient entre 0,010 et 57 mg/L. Onze composés distincts de plomb alkyle ont été identifiés dans les cadavres de ces poissons à des concentrations individuelles allant de quantités non décelables à 68,6 mg/kg. La somme de tous les composés de plomb alkyle dans les cadavres variait entre 0,24 et 144,5 mg/kg. On a noté de fortes corrélations entre le plomb total dans le sang, le plomb total dans le cadavre et la somme des composés de plomb alkyle dans le cadavre. Il y avait des poissons contaminés à 14 kilomètres en aval de la source, et dans certains cas à 16 kilomètres en amont, probablement en raison de la migration des poissons.

En 1982, des études ont confirmé une forte contamination par le plomb. Les carpes contenaient le plus de plomb alkyle, suivies du meunier noir, du grand brochet, de la perchaude et de la queue à tache noire. Le crapet de roche, la barbotte brune et l'achigan à petite bouche étaient parmi les espèces les moins contaminées. Des niveaux élevés de plomb ont été également trouvés dans les sédiments et les macrophytes, mais pas dans les myes, probablement en raison d'un panache distinct dans la rivière.

# TRACE METAL TOXICITY TO FISH IN ACID WATERS: MODIFICATION BY ORGANIC ACIDS AND ALKALIZATION

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HUTCHINSON, N.J. and J.B. SPRAGUE. 1985. Trace metal toxicity to fish in acid waters: modification by organic acids and alkalization. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 225-226.

The impact of acidification on fish reproduction can be related to increased levels of several trace metals in soft, acid water. The severity of metal toxicity to fish in a given lake may be predictable on the basis of organic carbon content of the lake water. Liming acid lakes to restore pH will also protect fish populations from metal toxicity.

Previous studies determined that, at pH 5.8, the threshold for reproductive failure of fish in acid lakes, low pH alone had no effect on any aspect of reproduction in American flagfish (Jordanella floridea). A mixture of Al-Mn-Fe-Ni-Zn-Cu-Pb however, caused total reproductive failure in soft (5.0 ppm), acid (pH 5.8) water, primarily due to mortality of larval fish.

A series of lethal exposures of larval flagfish showed that the toxicity of Al, Zn and Cu together was equivalent to that of the entire 7-metal mixture. At the metal ratios (13:5:1) occurring in acid lakes apparent additive toxicity of each component occurred.

Organic acids present in four lake waters reduced the metal toxicity, compared to that in manufactured soft water. A linear correlation of LC50 with T.O.C., ultraviolet absorbance (275 and 350 nm) and simple colour suggested that the mechanism was metal complexation with humic and tannic materials.

When lime was added to acid-lake microcosms in the laboratory, metal toxicity to larval fish was reduced by a factor of three. The effect appeared to be more related to changes in hardness and alkalinity than to changes in pH.

HUTCHINSON, N.J. and J.B. SPRAGUE. 1985. Trace metal toxicity to fish in acid waters: modification by organic acids and alkalization. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 225-226.

Les effets de l'acidité sur la reproduction des poissons peuvent être reliés à l'accroissement du niveau de divers métaux à l'état de trace dans l'eau douce acide. La gravité de la toxicité des métaux pour les poissons dans un lac donné peut être prédite en fonction de la teneur en carbone organique de l'eau de ce lac. La neutralisation de l'acidité des lacs en vue de rétablir le pH normal, peut également aider à protéger les populations de poissons de la toxicité métallique.

Des études antérieures ont permis de déterminer qu'au pH 5,8, seuil de la cessation de la reproduction des poissons dans les lacs acides, le pH faible, à lui seul, n'a pas d'effet sur la reproduction de l'American flagfish (Jordanella floridae). Un mélange des métaux Al-Mn-Fe-Ni-Zn-Cu-Pb a cependant causé la cessation totale de la reproduction en eau douce (5,0 ppm), acide (pH 5,8), essentiellement en raison de la mortalité des larves de poisson.

Une série d'expositions létales des larves de flagfish a montré que la toxicité de Al, Zn et Cu ensemble était équivalente à celle du mélange total des 7 métaux. Dans les proportions de métaux (13:5:1) qui se présentent dans les lacs acides, il semble que la toxicité soit la somme de la toxicité de chaque composant.

Les acides organiques présents dans l'eau de quatre lacs ont réduit la toxicité métallique, par comparison à celle de l'eau douce fabriquée. Une corrélation linéaire de CL50 avec le carbone organique total, l'absorption d'ultra-violets (275 et 350 nm) et la couleur simple semblent indiquer que le mécanisme en cause a été celui d'une formation complexe des métaux avec des produits humiques et tanniques.

Par addition de chaux au microcosme de lac acide en laboratoire, la toxicité des métaux pour les larves de poissons a été réduite par un facteur de trois. Cet effet semble relié à des modifications de la dureté et de l'alcalinité plutôt qu'à des modifications du pH.

# BEHAVIOR AND BIOLOGICAL EFFECTS OF ACIDIC INDUSTRIAL WASTE WATER CONTAINING HEAVY METALS

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LEHTINEN, K.J. 1985. Behavior and biological effects of acidic industrial waste water containing heavy metals. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 227-228.

Different biological and chemical methods have been used to characterize the behavior and biological effects of a complex, acid waste water. The toxicity of the total waste water was tested, as well as the toxicity of its soluble acid, non-soluble fractions. Neither the soluble or the non-soluble fraction proved to be biologically inactive. Water temperature was found to be the dominating factor regulating the solubility of metals present in the waste water at neutral pH with more metals in solution at low temperature than at high. This was illustrated by a higher acute toxicity to *Nitocra spinipes* Boeck at 4°C (96 h LC50: 870 ul/l) than at 21°C (96h LC50: 1200 ul/l). The role of the precipitative mechanisms for the toxicity of unfractionated waste water was illustrated by two fecondity minima of *N. spinipes*, one obtained at a concentration of 1000 ul/l (mainly mechanical action of precipitate) and another at 3-10 ul/l (no precipitate present). Total, unfractionated waste water caused hematological and biochemical disturbances in the flounder, *Platichtys flesus* (L.), in sublethal concentrations of waste water at low (4°C) water temperature. The soluble fraction of the waste water was shown to cause more serious toxic effets to the rainbow trout, *Salmo gairdneri* (R.) at low temperature than at high. Metal uptake from the soluble fraction into the gills of the perch, *Perca fluviatilis* (L.) was investigated at different water temperatures. In mucous cells of gills from fish exposed at 5°C Fe, Ti and Cu were detected, whereas only Cu was detected in these cells at 14-15°C. The non-soluble fraction had an acute 96h LC50 value c. 60 times higher than the original waste water when tested upon *N. spinipes*. The situation in the recipient is discussed in the light of two theoretical models based upon the results obtained and on previously known recipient data.

LEHTINEN, K.J. 1985. Behavior and biological effects of acidic industrial waste water containing heavy metals. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 227-228.

Différentes méthodes biologiques et chimiques ont été utilisées pour caractériser le comportement et les effets biologiques des eaux usées complexes acides. La toxicité de l'eau usée totale a été évaluée, ainsi que la toxicité des fractions soluble et non soluble. Ni la fraction soluble, ni la fraction non soluble ne se sont révélées biologiquement inactives. On a découvert que la température de l'eau était le facteur prédominant dans la régulation de la solubilité des métaux présents dans les eaux usées à pH neutre, avec une plus grande quantité de métaux en solution à basse température qu'à haute température. Comme exemple, citons la plus forte toxicité aigue pour *Nitocra spinipes* Boeck à 4°C (CL50 - 96h: 870 µl/l) qu'à 21°C (CL50 - 96H: 1 200 µl/l). Le rôle des

Mécanismes de précipitation dans la toxicité des eaux usées non fractionnées et illustré par deux minimums de fécondité de *N. spinipes*, l'un obtenu à la concentration de 1 000 µl/l (principalement l'action mécanique du précipité) et l'ature à 3-10 µl/l (sans précipité présent). Les eaux usées totales non fractionnées ont provoqué des troubles hématologiques et biochimiques chez la plie, *Platichtys flesus* (L.), à des concentrations sublétales d'eau usée à basse température (4°C). La fraction soluble des eaux usées causait des effets toxiques plus graves chez la truite arc-en-ciel, *Salmo gairdneri* (R.), à basse température qu'à haute température. La fixation de métaux à partir de la fraction soluble dans les branchies de la perchaude, *Perca fluviatilis* (L.), a été étudiée à différentes températures des eaux. Dans les cellules muqueuses des branchies, chez des poissons exposés à 5°C, on a pu déceler Fe, Ti et Cu, tandis qu'à 14-15°C, seul le cuivre a été décelé dans ces cellules. La fraction non soluble avait une valeur de CL50 à 96h d'environ 60 fois plus élevée que l'eau usée d'origine, testée sur *N. spinipes*. La situation chez le récepteur est analysée à la lumière de deux modèles théoriques fondés sur les résultats obtenus et sur des données antérieurement connues sur les récepteurs.

# MODEL ECOSYSTEMS IN MARINE ECOTOXICOLOGICAL RESEARCH IN SWEDEN

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LINDEN, O., K.-J. LEHTINEN, and M. NOTINI. 1985. Model ecosystems in marine ecotoxicological research in Sweden. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 229-230.

Model ecosystems simulating the littoral zone of the Baltic Sea have been developed as a test method in marine ecotoxicological research. Model ecosystems of the bladder wrack belt have been established in circular commercially available pools with a water volume of 7.7 $m^3$. The pools are provided with a flow of sea water of 2.5 l/min. Except for large predators all the normal components of the fauna and flora in the littoral zone are introduced into the pools. Macroscopic brown algae attached to stones are collected and these cover 1/3 of the bottom area of the pools. The rest of the bottom is left as an open sand bottom. The total number of macroscopic animals in each pool is estimated at $2 \times 10^4$ individuals of 30 species. In several experiments biological and physio-chemical parameters of the pools have been compared between different pools and with the natural ecosystem in a bay. The results have indicated extremely good stability between the pools and also a very good agreement between the parameters of the model ecosystem and those of the natural system. The model ecosystem have been used in several long-term experiments involving oil, oil + dispersants, and pulp mill effluents. Before any experiment the model ecosystems are allowed to stabilize for at least one month prior to start of the exposure. Experiments have been carried out for a period of up to 1 year. The paper gives a more detailed description of the experimental set up as well as a review of some representative results.

LINDEN, O., K.-J. LEHTINEN, and M. NOTINI. 1985. Model ecosystems in marine ecotoxicological research in Sweden. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 229-230.

Des modèles d'écosystèmes simulant la zone littorale de la mer Baltique ont été mis au point comme méthode d'essai pour la recherche en écotoxicologie marine. Des modèles d'écosystèmes du fucus vésiculeux ont été établis dans des piscines circulaires disponibles dans le commerce, avec un volume d'eau de 7,7 $m^3$. Ces piscines sont équipées d'une circulation d'eau de mer de 2,5 l/min. A l'exception des grands prédateurs, on a introduit dans les piscines tous les éléments normaux de la faune et de la flore de la zone littorale. Des algues brunes macroscopiques attachées à des pierres sont rassemblées et étendues sur le tiers de la surface du fond des piscines. Le reste est un fond de sable. Le nombre total d'animaux macroscopiques dans chaque piscine est estimé à $2 \times 10^4$ individus de 30 espèces. Dans plusieurs expériences, les paramètres biologiques et physicochimiques des piscines ont été comparés entre différentes piscines et avec l'écosystème naturel d'une baie. Les résultats ont indiqué une excellente stabilité entre les piscines, ainsi qu'un très bon accord entre les paramètres de l'écosystème modèle et ceux du système naturel.

Les modèles d'écosystèmes ont été utilisés dans plusieurs expériences à long terme avec le pétrole, le pétrole et des dispersants, et des effluents d'usine de pâte à papier. Avant toute expérience, les modèles sont laissés en repos pour qu'ils se stabilisent pendant au moins un mois.

Les expériences ont duré jusqu'à un an. La présentation donne une description plus détaillée de l'installation expérimentale, ainsi qu'un aperçu de quelques résultats représentatifs.

# AN EXAMINATION OF COPPER AND NICKEL CONCENTRATIONS IN SELECTED TISSUES OF RAINBOW TROUT

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MCCONNELL, A.S. and P.M. STOKES. 1985. An examination of copper and nickel concentrations in selected tissues of rainbow trout. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 231-232.

Rainbow trout (*Salmo gairdneri* Richardson) were exposed to a series of 4 nickel treatments (4, 8, 16, 32 ppm), 2 copper treatments (1 and 5 ppm) and 1 copper-nickel mixture (1 ppm/8 ppm) in water of approximately 100 mg/l hardness (as $CaCO_3$), 85 mg/l alkalinity, a pH of 7.5 and temperature of 10°C.

In a period of 96 hours, no mortality was observed in the nickel treatments. However, 100% mortality occurred at 77 hours and 10 hours in the copper treatments respectively and at 30 hours in the copper-nickel mixture.

Two interesting developments were observed in the nickel treatments: as nickel concentrations increased the nickel content of the gills increased but the gill copper concentration decreased. At the same time, while the nickel concentration of the liver only increased significantly from the control fish at the higher nickel treatment, the copper concentration of the liver increased through all the nickel treatments even though these fish had not been experimentally exposed to copper.

The fish exposed to copper treatments alone showed a significant increase in the copper concentration of the gill as well as an increase in the copper concentration in the liver.

The fish in the copper-nickel mixture, when compared to those exposed to a 1 mg/l copper treatment, exhibited no increase in the copper concentration of the liver and there was no significant difference in the copper concentration of the gills of these fish.

The fish treated in the copper-nickel mixture showed a significant increase in the copper concentration of the gills compared to control fish but displayed no difference when compared to fish exposed to the same copper treatment in the absence of nickel. There were also increased nickel concentrations in the gill and liver of fish in the copper-nickel mixture. When compared to nickel alone, the copper-nickel treatment showed a significant increase in the concentration of nickel in the gill but the increase of nickel in the liver was not significant.

It is suggested that in the "nickel alone" treatment, existing copper may be replaced by nickel at the gill and that this may be one method of detoxification. However, in the presence of added copper, as in the copper-nickel mixture, nickel uptake may be enhanced at the gill causing a large increase of nickel into the fish's systemics, potentially blocking the uptake of copper into the liver. This could account for a significantly faster death rate.

MCCONNELL, A.S. and P.M. STOKES. 1985. An examination of copper and nickel concentrations in selected tissues of rainbow trout. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 231-232.

Des truites arc-en-ciel (Salmo gairdneri Richardson) ont été exposées à une série de quatre traitements au nickel (4, 8, 16, 32 ppm), à deux traitements au cuivre (1 et 5 ppm) et à un traitement au mélange cuivre-nickel (1 ppm/8 ppm) dans de l'eau d'une dureté d'approximativement 100 mg/L ($CaCO_3$), d'un alcalinité de 85 mg/L, d'un pH de 7,5 et d'une température de 10°C.

Pendant une période de 96 heures, on n'a observé aucune mortalité avec le traitement au nickel. Cependant, le taux de mortalité était de 100% après 77 heures et 10 heures, respectivement, pour les traitements au cuivre, et après 30 heures de traitement au mélange cuivre-nickel.

Deux événements intéressants ont été observés avec les traitements au nickel: à mesure qu'augmentaient les concentrations en nickel, le contenu en nickel des branchies augmentait également, tandis que la concentration en cuivre des branchies diminuait. En même temps, pendant que la concentration en nickel seul dans la foie augmentait de façon significative par rapport aux poissons témoins avec le traitement au nickel à dose plus élevée, la concentration en cuivre du foie augmentait pendant tout le traitement au nickel, bien que le poisson n'ait pas été exposé expérimentalement au cuivre.

Le poisson exposé au traitement de cuivre seul a montré une augmentation significative de la concentration de cuivre des branchies, ainsi qu'une augmentation de la concentration de cuivre du foie.

Le poisson exposé au mélange cuivre-nickel, par comparaison à celui qui était exposé à 1 mg/L de cuivre, n'a pas subi d'augmentation de sa concentration en cuivre au niveau du foie, ni n'a présenté de différence significative au niveau des branchies.

Chez le poisson traité au mélange cuivre-nickel, on a noté une augmentation significative de la concentration de cuivre au niveau des branchies, par comparaison aux poissons témoins, mais aucune différence, par comparaison avec le poisson exposé au même traitement de cuivre en l'absence de nickel. On a noté également une concentration de nickel accrue au niveau des branchies et du foie des poissons soumis au mélange cuivre-nickel. Comparé au nickel seul, le traitement cuivre-nickel a entraîné une augmentation significative de la concentration de nickel dans les branchies, mais l'augmentation du nickel dans le foie n'a pas été significative.

Ces résultats semblent indiquer que dans le traitement au nickel seul, le cuivre existant peut être remplacé par le nickel au niveau des branchies, ce qui peut représenter une méthode de détoxification. Cependant, en présence de cuivre surajouté, comme dans le mélange cuivre-nickel, la fixation de nickel peut être renforcée au niveau des branchies, provoquant une importante augmentation du nickel dans le système général du poisson, et pouvant bloquer la fixation de cuivre dans le foie. Ces divers phénomènes pourraient jouer un rôle important dans l'accélération du taux de mortalité.

# MECHANISMS OF ALUMINIUM TOXICITY IN MODERATELY ACIDIC CONDITIONS TO SALMONIDS

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VAN COILLIE, R., C. THELLEN, R. ROY, and Y. VIGNEAULT. 1985. Mechanisms of aluminium toxicity in moderately acidic conditions to salmonids. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 233-234.

The release of aluminium caused by acidic precipitation into the waters of the Canadian Shield becomes an increasing interest. In this context, we have studied the histo-physiological and biochemical effects brought on by this metal at pH 4,5 and 5,5 in very soft water on Salmo salar and Salvelinus fontinalis (age 1+) using 7 day flow through continuous flow bioassays. The chemical speciation of aluminium has also been determined in the course of these bioassays. This metal preferentially accumulates in the gills, where after 7 days it is found at levels up to 25 times higher than those in the milieu. Aluminium provokes impairments in the gills (desquamation, bubbling, etc.) in addition to those caused by acidity. This metal amplified too the physiological effects on these tissues, as for hyperventilation, increased oxygen consumption or hypersecretion of mucus. Aluminium induces an increase in RNA and protein synthesis, not only in the gills but also in the liver. This internal increase of protein synthesis depends on the degree of penetration of aluminium, the hormonal feedback, the macromolecular response to metallic aggression and the fixation sites of aluminium. (X-Ray dispersive wavelength microanalysis in electron microscopy).

VAN COILLIE, R., C. THELLEN, R. ROY, and Y. VIGNEAULT. 1985. Mechanisms of aluminium toxicity in moderately acidic conditions to salmonids. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 233-234.

La libération d'aluminium provoquée par les précipitations acides dans les eaux du Bouclier canadien suscite un intérêt grandissant. Dans ce contexte, nous avons étudié les effets histophysiologiques et biochimiques provoqués par ce métal aux pH 4,5 et 5,5 en eau très douce sur *Salmo salar* et *Salvelinus fontinalis* (1+ an) au cours de bio-essais en flot continu d'une durée de 7 jours. La spécification chimique de l'aluminium a également été déterminée au cours de ces études biologiques. L'aluminium s'accumule de préférence dans les branchies où, 7 jours après, on le trouve à des niveaux parfois 25 fois supérieurs à ceux du milieu. L'aluminium provoque des lésions au niveau des branchies (desquamation, formation de bulles, etc.) en plus de celles que cause l'acidité. L'aluminium amplifie également les effets physiologiques sur ces tissus: hyperventilation, augmentation de la consommation d'oxygène et hypersécrétion de mucus. L'aluminium induit un accroissement de la synthèse du RNA et des protéines, non seulement dans les branchies, mais également dans le foie. Cette augmentation interne de la synthèse des protéines dépend du degré de pénétration de l'aluminium, de la réaction hormonale et de la réponse

macromoléculaire à l'agression métallique, ainsi que des lieux de fixation de l'aluminium (microanalyse aux rayons X à longueur d'ondes dispersives en microscopie électronique).

# BIOLOGICAL AND CHEMICAL METHYLATION OF LEAD COMPOUNDS IN THE AQUATIC ENVIRONMENT

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WONG, P.T.S. and Y.K. CHAU. 1985. Biological and chemical methylation of lead compounds in the aquatic environment. Can. Tech. Rep. Fish. Aquat. Sci. 1368: p. 235.

Since the observation that mercury could be methylated into a highly toxic methylmercury compound in a natural aquatic environment, a tremendous amount of interest has been generated on the methylation of other elements. Our laboratory was the first to report in 1975 that microorganisms in certain lake sediments could methylate certain inorganic Pb (II) and organic Pb (IV) compounds into a volatile and highly toxic tetramethyllead ($Me_4$ Pb). Many papers have subsequently been published by various investigators on this important topic with contradictory results and interpretations. It is now generally accepted that Pb (IV) can be methylated to $Me_4$ Pb via biological and chemical reactions. However there is some debate on the possibility of $Pb^{2+}$ methylation. The various theories on this interesting and important lead methylation process will be discussed.

WONG, P.T.S. and Y.K. CHAU. 1985. Biological and chemical methylation of lead compounds in the aquatic environment. Can. Tech. Rep. Fish. Aquat. Sci. 1368: p. 235.

Depuis qu'on a observé que le mercure peut être méthylé et former ainsi un composé de méthylmercure extrêmement toxique pour l'environnement aquatique naturel, l'intérêt des chercheurs s'est porté sur la méthylation des autres éléments. Notre laboratoire a été le premier à signaler en 1975 que des microorganismes localisés dans certains sédiments lacustres étaient capables de méthyler certains produits inorganiques de plomb Pb (II) et organiques de plomb Pb (IV), et de donner ainsi du plomb tétraméthyle, instable et extrêmement toxique ($Me_4$ Pb). Par la suite, de nombreuses publications ont été consacrées à ce sujet important, donnant souvent des résultats et des interprétations contradictoires. Il est maintenant généralement accepté que le plomb (IV) peut être méthylé en $Me_4$ Pb par des réactions biologiques et chimiques. La possibilité de méthylation de $Pb^{2+}$ n'est cependant pas encore tout à fait admise. Nous analyserons les diverses théories actuellement en présence, concernant le processus de la méthylation du plomb, sujet à la fois important et intéressant.

# ORGANOHALOGENS AND ENVIRONMENTAL PHYSIOLOGY

Roy Parker, Chairman

# REFERENCE MATERIALS FOR THE DETERMINATION OF PCBs AND PAHs IN MARINE SEDIMENTS

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GUEVREMONT, R., W.D. JAMIESON, and E. Lewis. 1985. Reference materials for the determination of PCBs and PAHs in marine sediments. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 237-247.

Eight sets of marine sediment reference materials have been prepared, three for the determination of PCBs and five for PAHs. The samples are unspiked natural sediments from locations chosen to present a range of analyte concentrations and matrix types. Semiquantitative measurements of PCBs by packed-column GC/ECD and quantitative determinations of ten PCB congeners by capillary-column GC/ECD ad GC/MS are described. HPLC, capillary-column GC/FID and GC/MS techniques have been applied to the quantitative determination of sixteen PAH compounds. Mass spectrometric measurements to identify and quantitate both PCBs and PAHs are discussed. A project in progress to prepare reference solutions of PCB congeners is described.

GUEVREMONT, R., W.D. JAMIESON, and E. Lewis. 1985. Reference materials for the determination of PCBs and PAHs in marine sediments. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 237-247.

Huit ensembles de documents de référence sur les sédiments marins ont été préparés, dont trois pour la détermination des BPC et cinq pour celle des hydrocarbures aromatiques polycycliques (HAP). Les échantillons sont des sédiments naturels non altérés, provenant d'endroits choisis pour présenter une gamme de concentrations de produits analysés et de types de matrices. On décrit des mesures semi-quantitatives du taux de BPC au moyen de la chromatographie en phase gazeuse et du détecteur à capture électronique sur colonne compacte, et des déterminations quantitatives de dix congénères BPC par chromatographie en phase gazeuse et détecteur à capture électronique sur colonne capillaire ainsi que par chromatographie en phase gazeuse et spectrophotométrie de masse. Les techniques de chromatographie liquide à haute pression, de chromatographie en phase gazeuse et détection par ionisation de flamme et de chromatographie en phase gazeuse et spectrophotométrie de masse sur colonne capillaire ont été appliquées à la détermination quantitative de seize composés HAP. Les mesures spectrométriques destinées à identifier et quantifier à la fois BPC et HAP sont analysées. Nous décrivons enfin un projet en cours destiné à préparer des solutions de référence de congénères BPC.

## INTRODUCTION

Hydrophobic organic components introduced into the marine environment generally associate with particulates in the water and eventually are found in marine sediments. From the sediments they may be introduced into the biota or be resuspended in the water column by biological activity. Sediment materials are also transported by the actions of waves and currents. Components of the sediments are continually subject to chemical change initiated biochemically or by such physico-chemical factors as the absorption of light or changes in redox potential. Analytical capability adequate to reliably identify and quantitate such organic components is essential to the understanding of their role in the marine environment.

Commercial mixtures of chlorinated biphenyls (PBCs) have been widely used in industry since 1929 as heat transfer fluids, dielectric or hydraulic fluids, flame retardants, solvent extenders and plasticizers. Their uses are due to such physical and chemical properties as resistance to acids and bases and to oxidation or reduction, along with excellent thermal stability and non-flammability. These industrially valuable characteristics unfortunately also contribute to the persistance of PCBs in the environment.

Deliberate and accidental disposals of PCBs have led to worldwide, significant contamination of the environment[1]. Due to their stability and strong tendency to be bioaccumulated, PCBs have entered the food chain and are commonly found in human adipose tissue. Their toxicity is widely recognized and it has been proposed that they be regarded as carcinogenic to humans. Although in recent years many countries have closely regulated the manufacture and use of PCBs, disposal of waste PCBs is a continuing source of environmental contamination and long-term human exposure is inevitable.

The properties and uses of commercial PCBs, produced by direct chlorination of biphenyl, depend on the degree of chlorination. The PCB products used are complex mixtures of various isomers and congeners[2,3]. Of the 209 possible individual congeners, more than fifty are contained in most commercial products. Differences between the populations of compounds present in these products depend on the degree of chlorination and the production batch.

Determinations of trace levels of PCBs in marine samples are done frequently to serve regulatory needs and to gain research data. The accuracy of these analytical data has often been questioned. Determinations of PCBs and assessment of the toxicological significance of such data are very difficult because of the large number of compounds present in commercial mixtures, wide variance in the toxicity of individual compounds and differences between the populations of compounds in residues being analyzed and the parent commercial products. Although advances in analytical methods such as measurement by high-resolution gas chromatography now allow precise and specific data to be obtained, suitable reference materials and standards have not been available to assure accuracy and aid in the comparison of results from different analytical laboratories or differing analytical methods.

The Marine Analytical Chemistry Standards Program (MACSP) of the National Research Council of Canada has made available three sets of marine sediment reference materials in which the natural concentrations of PCBs present have been determined reliably. The semiquantitative total PCBs content (taken as "Aroclor 1254") is reported for these samples. The concentrations of ten individual PCB congeners in two of these reference materials have also been determined.

Polycyclic aromatic hydrocarbons (PAHs) are widely dispersed in the environment, usually due to the use of fossil fuels. Though PAHs probably enter the marine environment mainly through air and river discharges, the hazards to the fishery associated with off-shore petroleum exploration and development must be assessed to determine whether there might be significant damage from that source. In common with other hydrophobic materials such as PCBs, PAHs associate with particulates in the water column and eventually find their way to the sediments. Unlike PCBs, PAHs are much more subject to chemical changes induced biologically or geochemically. Though such changes result in degradation of PAHs to harmless products, they also produce many xenobiotic compounds of differing toxicity. It is difficult to determine accurately the concentrations of these compounds and to assess their effects on the marine environment.

The MACSP has developed a series of five marine sediment reference materials for which the concentrations of up to sixteen PAHs present naturally have been determined reliably. Samples of three or four of these materials will be released for distribution soon.

## Preparation

The preparation methods used were developed to yield a homogeneous, uncontaminated product for which the effects of sample matrix on the analysis would closely resemble such effects of the original source sediment. To date, all bulk samples processed were collected from Eastern Canadian coastal sites ranging from busy commercial harbours to uncontaminated open-ocean areas.

Collected samples are freeze-dried, then sieved to pass 100 mesh screens before being blended thoroughly in a modified, electrically powered concrete mixer. During blending, the adequacy of mixing is assessed by analysis of sub-samples. After a sample has been homogenized, the final series of sub-samples is made. These final sub-samples are transferred as they are taken to previously cleaned steel cans of the type used commercially to contain paint, then sealed immediately to exclude air. The closure used is the usual steel cover which has no organic components.

Multiple sub-samples from a series of cans selected randomly are analyzed to assess intra- and inter-can homogeneity of the prepared material and to establish reliably the concentrations of specific analytes.

## Analysis

As usually defined in analytical chemistry, a reference material is a material which emulates the chemical characteristics of samples being analyzed and for which it is reliably known that sub-samples will contain (within defined limits) stated concentrations of a specified group of analytes[4,5].

It is difficult to establish reliable values for the concentrations of low to trace levels of analytes such as PCBs or PAHs in complex natural samples such as sediments. The preferred methodology would use at least two completely independent analytical methods. However, any sediment sample analyzed for trace levels of organic compounds must be extracted with solvent, thus partitioning the sample and associating with measured concentration values uncertainties due to extraction efficiencies. The simple, unambiguous concept of "total analysis" used in elemental analysis cannot be applied since there is no way to make analytical measurements directly on the whole sample or a derivative such as a digest which represents all of it. Though corroborative analyses in trace organic analysis cannot be truly independent, they must be chosen to be as different and as specific as possible. Confidence in concentrations determined can be improved by comparing results obtained by different laboratories which have appropriate expertise if it has first been established that samples to be used are closely similar and stable.

For the marine sediment reference materials CS-1, HS-1 and HS-2 concentrations of total PCBs were measured by electron-capture detection (ECD) following low resolution packed-column gas chromatography[6]. Although results from this technique must be considered semiquantitative at best, they are useful to many laboratories who must measure PCB contents this way. Results we obtained (Table 1) were in acceptable agreement with those obtained by other laboratories. Documentation describing the analytical procedure is supplied with samples distributed. A typical low-resolution chromatogram is shown in Figure 1.

When higher-resolution capillary-column gas chromatography is used to separate PCBs, the concentrations of individual PCB congeners can usually be determined, although some of the chromatographic peaks may still be due to more than one congener[2,3]. A typical high-resolution chromatogram is shown in Figure 2. Measurement was by electron-capture detection.

Capillary-column GC/ECD, capillary-column GC/MS using negative ion data from chemical ionization[7] and proton magnetic resonance data were used to identify and quantitate ten PCB congeners (IUPAC numbers[2] 101, 138, 151, 153, 170, 180, 194, 196, 201, 209) in the marine sediment reference materials HS-1 and HS-2. Concentrations of PCB in the CS-1 material were too low for reliable determination of the concentrations of individual congeners. Quantitative data will be available after results from corroborative analyses are complete.

In our laboratory, mass spectrometry is used wherever possible for corroborative qualitative or quantitative determinations. High-resolution mass spectrometry with photographic plate detection has long been a favoured technique for the identification and semiquantitative determination of trace levels of xenobiotic compounds in crude extracts of biological tissue and other complex materials without much "clean-up" prior to analysis[8,9,10,11]. Artifacts and interferences are usually minimum and the specificity of the determination is aided by significant, negative mass defects for isotopes usually present in xenobiotics. Less specific low-resolution mass spectrometry still yields multidimensional data and gains specificity as such measurements are coupled directly to mixture separation techniques such as capillary gas chromatography or HPLC. Appropriate choices of ionic detection methods (e.g., positive or negative ions, monitoring ions of selected mass or mass ranges) and sample ionization methods (e.g., electron impact or chemical ionization with optimum reagent gas mixtures) adds more selectivity and sensitivity[12]. Thus, organohalogen compounds such as PCBs are often best determined by

TABLE 1 DETERMINATION OF PCB IN HS-1, HS-2, AND CS-1

| Determination[2] | PCB[1] (μg/kg dry sediment) CS-1 | HS-1 | HS-2 |
|---|---|---|---|
| 1 | 0.95 | 20.6 | 109.6 |
| 2 | 0.54 | 20.4 | 108.5 |
| 3 | 0.81 | 23.3 | 110.0 |
| 4 | 1.96 | 22.0 | 115.2 |
| 5 | 2.17 | 23.2 | 112.5 |
| 6 | 0.62 | 23.0 | 110.4 |
| 7 | 0.98 | 21.6 | 114.1 |
| 8 | 1.16 | 20.7 | 111.4 |
| 9 | | 22.0 | 115.9 |
| 10 | | 20.8 | 110.5 |
| Average ± Standard Deviation | 1.15<br>± 0.60 | 21.8<br>± 1.12 | 111.8<br>± 2.5 |

[1] Taken as Aroclor 1254 (Analabs, Inc., Lot J147A).

[2] Each determination refers to a minimum of 4 extractions and GC quantitation carried out on one can of marine sediment.

negative-ion chemical ionization since an appropriate reagent gas yields much more specific data than does electron impact ionization while matrix effects are minimized[7]. We have also found quantitation by mass spectrometry has the advantage of allowing the use as internal standards of isotopically labelled additions of the compounds being determined. Yet more specific, more sensitive determinations are possible by tandem mass spectrometry[13,14].

## Work in Progress

In further support of the determination of individual PCB congeners, a series of four iso-octane solutions of mixtures of selected, pure PCB congeners at stated concentrations will be available from the MACSP in 1984. Most of the 51 congeners listed in Table 2 will be included in one or more of these solutions. Congeners will be chosen for inclusion in each solution so that each mixture should be readily separable using a relatively inefficient 12-15 m capillary column. Each solution will contain compounds 15, 153 and 209 (IUPAC numbers[2]) for inter-solution comparisons. Where possible a pair of closely eluting compounds such as IUPAC numbers 196 and 201 will be included in a solution to aid testing the resolution of the chromatographic column.

Analyses of the further series of marine sediment reference materials to determine concentrations of PAHs is in progress. Capillary-column GC/FID (flame ionization detection), and capillary-column GC/MS have been used to obtain reliable data for the concentrations of sixteen PAH compounds. For corroboration, we have also used high pressure liquid chromatography (HPLC) with ultraviolet and fluorescence dectection to

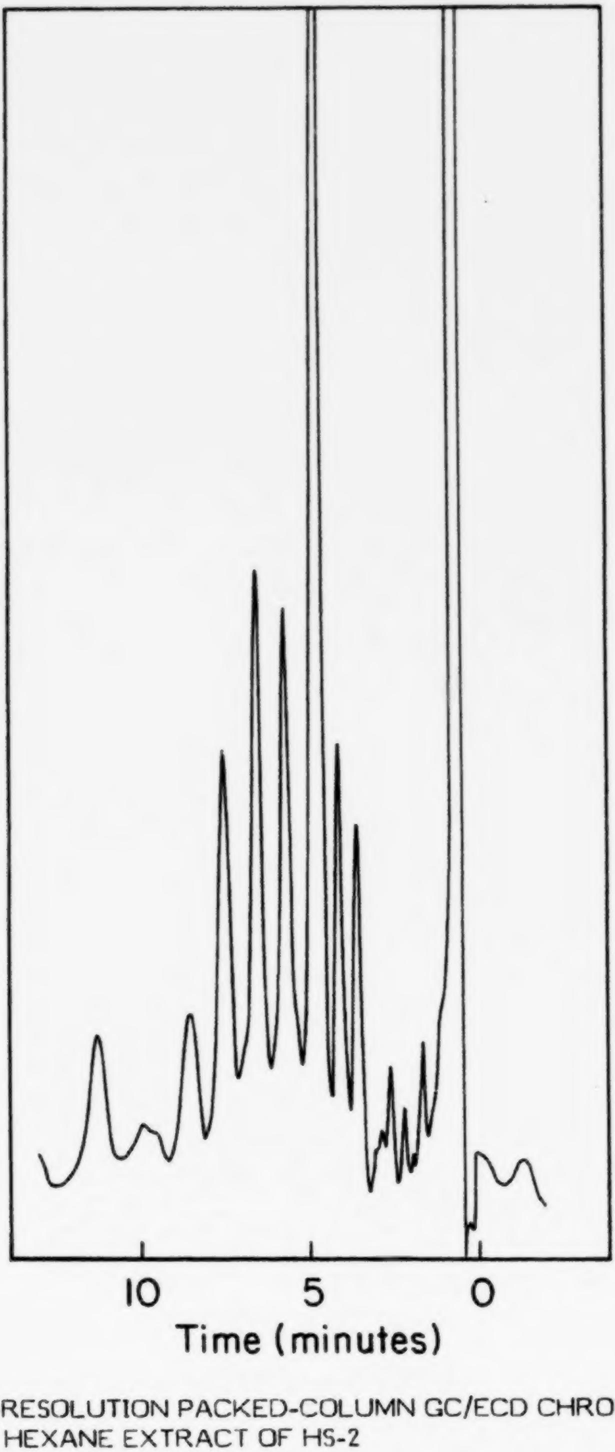

FIGURE 1 LOW-RESOLUTION PACKED-COLUMN GC/ECD CHROMATOGRAM OF A HEXANE EXTRACT OF HS-2

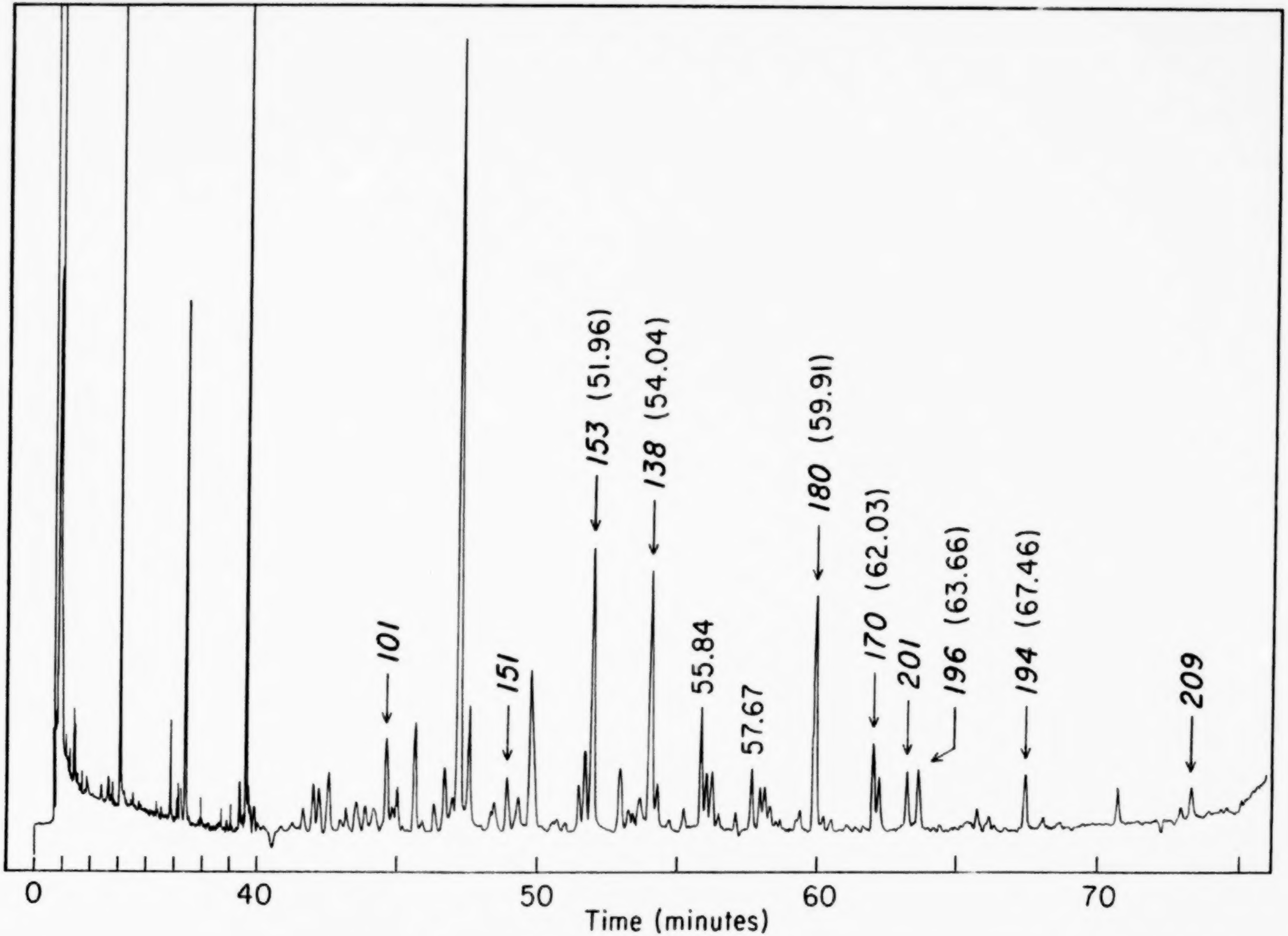

FIGURE 2 CAPILLARY-COLUMN GC/ECD CHROMATOGRAM OF A HEXANE EXTRACT OF HS-2 WITH SEVERAL PCB CONGENERS IDENTIFIED (IUPAC NUMBERS[2])

TABLE 2 PCB CONGENERS TO BE INCLUDED IN REFERENCE MIXTURES OF PCBS IN ISO-OCTANE

| IUPAC number | Chlorination | IUPAC number | Chlorination | IUPAC number | Chlorination |
|---|---|---|---|---|---|
| 15 | $Cl_2$ | 128 | $Cl_6$ | 194 | $Cl_8$ |
| | | 129 | | 195 | |
| 18 | $Cl_3$ | 137 | | 196 | |
| 31 | | 138 | | 200 | |
| | | 141 | | 201 | |
| 40 | $Cl_4$ | 143 | | 202 | |
| 44 | | 151 | | 203 | |
| 49 | | 153 | | 205 | |
| 52 | | 154 | | | |
| 54 | | 156 | | 206 | $Cl_9$ |
| 60 | | 159 | | 207 | |
| 77 | | | | 208 | |
| | | 170 | $Cl_7$ | | |
| 86 | $Cl_5$ | 171 | | 209 | $Cl_{10}$ |
| 87 | | 173 | | | |
| 101 | | 180 | | | |
| 103 | | 182 | | | |
| 105 | | 183 | | | |
| 114 | | 185 | | | |
| 118 | | 187 | | | |
| 121 | | 189 | | | |
| 126 | | | | | |

analyze these materials. As we have been able to use it, HPLC lacks sufficient resolution for reliable quantitative data to be obtained by measurement with such unidimensional detectors. Some HPLC/MS experiments done with an HPLC/MS interface we built[15] showed mass spectrometric measurement might improve the quality of the HPLC data enough for it to be quantitatively corroborative. Further HPLC/MS analyses are being done.

The series of four marine sediment reference materials we expect to make available in 1984 contain PAHs at concentrations ranging from below 0.1 mg/kg dry weight for compounds such as acenaphthalene and fluorene in a relatively "clean" material to levels in excess of 50 mg/kg dry weight for phenanthrene and fluoranthene in material from a more polluted area. Compound for which reliable concentration values will be stated include naphthalene, acenaphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(a)pyrene, benzo(ghi)perylene, dibenzo(ah)anthracene and indeno(1,2,3-cd)pyrene. Some oxygenated or nitrated derivatives of PAHs in these sediments may also be determined. Shown in Figure 3 is a typical capillary-column GC/FID chromatogram of an extract from one of the proposed reference materials which contains relatively high levels of PAHs.

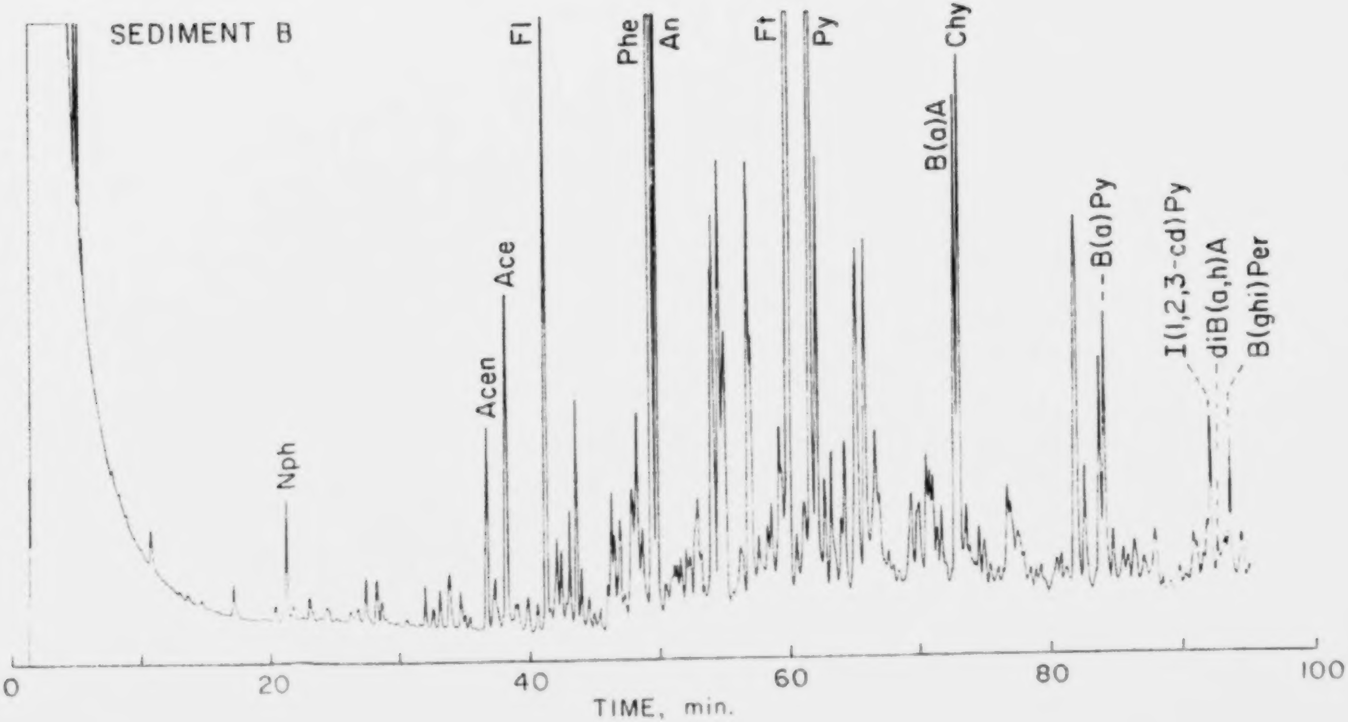

FIGURE 3 CAPILLARY-COLUMN GC/FID CHROMATOGRAM OF A HEXANE EXTRACT OF A PROPOSED PAH MARINE SEDIMENT REFERENCE SAMPLE. SEVERAL PAH COMPOUNDS ARE IDENTIFIED

## SUMMARY

Reference materials for determinations of PCBs and PAHs in marine sediments have been prepared. Many laboratories are already using the CS-1, HS-1 and HS-2 reference materials for determination of PCBs. These have been available for distribution since 1982. Data on the concentrations of ten individual PCB congeners in these materials will soon be released.

A series of marine sediment reference materials for determinations of up to 16 PAH compounds and a series of standard solutions to support determinations of up to 51 individual PCB congeners will be available for distribution in 1984.

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# TOXIC SUBSTANCES IN LAKE ST. FRANCIS SEDIMENTS

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SLOTERDIJK, H.H. 1985. Toxic substances in Lake St. Francis sediments. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 249-264.

Lake St. Francis is formed by a widening of the St. Lawrence River after entering the Quebec Province at Cornwall, and is potentially one of the most contaminated waterbodies in Quebec. In order to determine whether the lake acts as a sink for toxic substances coming from Lake Ontario and the international section of the St. Lawrence River, a bottom sediment survey was carried out during 1979-1981 at about a hundred stations. The first 2-3 cm were analysed for particle size, mercury and PCB's. About 30 stations were also analysed for PAH's, while 10 were analysed for mirex, chlorobenzenes, pentachlorophenol and PBB's.

Only PAH's, PCB's and Hg were found at significant concentrations, up to 1800, 1900 and 1500 ng/g, respectively. Distribution patterns indicate that Lake Ontario has less of an impact on the Quebec part of the St. Lawrence River than was previously suspected. Local sources such as Cornwall (Hg) and the New York tributaries of the international section of the St. Lawrence (PCB's, PAH's) are most likely responsible for the high contaminant levels in the lake. Nevertheless, Lake Ontario remains a significant source of contaminants (PCB's, mirex) for the Quebec region.

Particle size distribution suggests that Lake St. Francis is governed more by fluvial than lacustrine conditions, sand being the predominant fraction. The lake does not form a contaminant sink, and an important fraction of toxic substances will move through the system out towards the Montreal area.

There is an urgent need for the development and application of bioassays sensitive enough to evaluate the significance and ecotoxicological aspects of contaminant levels in sediments.

SLOTERDIJK, H.H. 1985. Toxic substances in Lake St. Francis sediments. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 249-264.

Le lac Saint-François, formé par un élargissement du fleuve Saint-Laurent après son entrée au Québec à Cornwall, est potentiellement un des plans d'eau les plus contaminés au Québec. Environ cent échantillons de sédiments de fond y ont été prélevés entre 1979 et 1981 afin d'évaluer le rôle du lac comme zone d'accumulation de substances toxiques provenant du lac Ontario et du tronçon international du fleuve Saint-Laurent. La couche supérieure (2-3 cm) des sédiments a été analysée pour la granulométrie, le mercure et les

PCB. Environ trente stations ont aussi été analysées pour les PAH, tandis que dix ont été analysées pour les chlorobenzènes et les PBB.

Seuls, les PAH, les PCB et le mercure ont été détectés à des concentrations significatives, soit jusqu'à 1800, 1900 et 1500 ng/g respectivement. Le patron de distribution spatiale indique que l'impact du lac Ontario sur la partie québécoise du fleuve Saint-Laurent est moindre que ce que l'on soupçonnait auparavant. Ce sont plutôt des sources locales, telles que Cornwall (Hg) et les tributaires new-yorkais du tronçon international du fleuve Saint-Laurent (PCB, PAH), qui sont responsables des niveaux élevés de contaminants dans le lac. Néanmoins, le lac Ontario demeure une source significative de substances toxiques (PCB, mirex) pour la région du Québec.

La granulométrie suggère que le lac Saint-François est plutôt caractérisé par des conditions fluviales que lacustres, le sable étant la fraction prédominante. Le lac ne forme pas une aire d'accumulation de contaminants et une importante fraction des substances toxiques sera acheminée en dehors du système vers la région de Montréal.

Il y a un besoin urgent pour le développement et l'application des tests biologiques assez sensibles pour évaluer la signification et les aspects écotoxicologiques des niveaux de contaminants dans les sédiments.

# INTRODUCTION

Lake St. Francis is formed by a widening of the St. Lawrence River after entering the Province of Quebec at Cornwall (Figure 1). It receives the waters of the Great Lakes by way of the international section leaving Lake Ontario (Kingston - Cornwall), and drains therefore one of the most industrialized and urbanized areas of North America. Lake St. Francis can be considered as potentially one of the most contaminated waterbodies in Quebec. This has been confirmed by several studies (CESL, 1978). For example, Sloterdijk (1977) has found high concentrations of Hg and PCB's in fish, while Sérodes (1978) reported high levels of mercury in sediments.

In order to determine whether Lake St. Francis acts as a sink for toxic chemicals coming from the Great Lakes, and especially Lake Ontario, a sediment survey was carried out in 1979, 1980 and 1981. Some of the results of this survey (Hg, PCB's and PAH especially) will be discussed in this report.

## Materials and Methods

About a hundred stations, located throughout the lake following roughly a grid system, were sampled during 1979, 1980 and 1981. Offshore stations were located using tellurometers (MRB 201) as described by Durette and Zrymiak (1978). Nearshore stations could be located using visual methods and compass readings.

Sediments were sampled using an Ekman dredge (15 x 15 cm) for the nearshore stations, and a Shipek (6600 $cm^3$) for the offshore stations.

The first 2-3 cm were taken and subsequently analysed for particle size, mercury and PCB's (Polychlorinated Biphenyls). About 30 stations were also analysed for PAH's (PolyAromatic Hydrocarbons) while 10 were analysed for mirex, chlorobenzenes, pentachlorophenol and PBB's (PolyBrominated Biphenyls).

Samples were preserved by freezing, and chemical analyses were carried out on the wet samples. Water contents was also determined and concentrations are reported on a dry weight basis. Particle size was determined by sieve, short pipette and settling tube analysis (Guy, 1969; Duncan and LaHaie, 1979). The fractions obtained represent: gravel, 2 mm; sand, 2 - 0.060 mm; silt, 0.060 - 0.004 mm; clay, 0.004 mm.

Mercury was determined by flameless atomic absorption spectrophotometry, after acid digestion and a permanganate/persulfate oxidation of the sediments. Reducing the mercury to its elementary form by stannous chloride permits air volatilization and subsequent measurement in an AA cell (Environment Canada, 1979).

Organic contaminants were determined using sediment extracts. PCB's, mirex, chlorobenzenes, pentachlorophenol and PBB's were analysed by gas-liquid chromatography (Environment Canada, 1979), while PAH's were analysed by High Pressure Liquid Chromatography (HPLC) according to a method described by Dunn and Armour (1980).

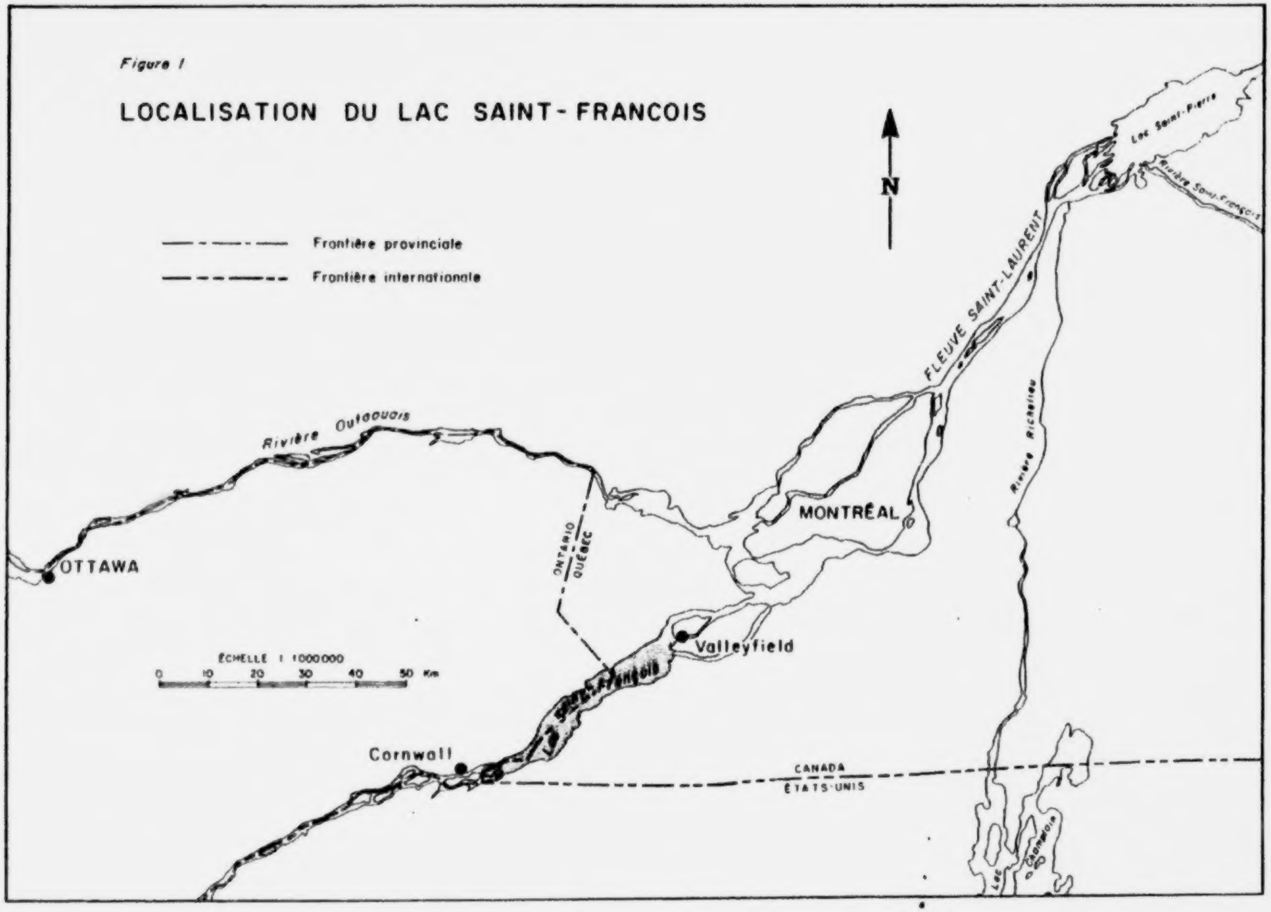
Figure I
LOCALISATION DU LAC SAINT-FRANCOIS
N
Frontière provinciale
Frontière internationale
Lac Saint-Pierre
Rivière Saint-François
FLEUVE SAINT-LAURENT
Rivière Richelieu
Rivière Outaouais
MONTRÉAL
ONTARIO
QUÉBEC
OTTAWA
ÉCHELLE 1:1000000
0 10 20 30 40 50 Km
Valleyfield
Lac Saint-François
Cornwall
CANADA
ÉTATS-UNIS
Lac Champlain

FIGURE 1 LOCALISATION DU LAC SAINT-FRANCOIS

# RESULTS AND DISCUSSIONS

## Study Area

To understand the lake and its hydrodynamics, some of its physical aspects should be mentioned. The lake forms a reservoir of about 230 km$^2$, with a length of about 60 km, and a width attaining a maximum of about 8 km. Hydro-electric dams upstream and downstream regulate its water level, which varies less than 0.5 m over a year.

There is no thermal stratification, the lake being fairly shallow. A significant current system ensures a short residence time of the water. The lake is also partially canalized for the St. Lawrence Seaway.

There are two major urbanized communities, one at each pole of the lake, Cornwall and Valleyfield. The Cornwall area is particularly industrialized and forms a potential source of contaminants, since it is located at the upstream end of the lake. As was mentioned before, Lake Ontario and the international section of the St. Lawrence River are important potential sources of toxic chemicals. The perimeter of the lake consists of a few villages and agricultural fields while the lake's tributaries drain an essentially agricultural region.

After the lake, the St. Lawrence River carries on by way of the Beauharnois canal towards Lake St. Louis and the Montreal area.

## Particle Size

Results for particle size distribution are shown in Figure 2. This parameter is important, since it influences contaminant absorption. In general, smaller particles (e.g. clay) adsorb toxic chemicals to a greater extent than larger particles (e.g. sand), for reasons of chemical affinity and volume/surface area ratio. Organic carbon content also plays a role, and it is thought that PCB's, for example, are concentrated by organic matter in the sediments.

Throughout the lake we found mainly sand. The reason for this predominance of sand is thought to be due to the fluvial character of the lake. Being a widening of the St. Lawrence River, typical lacustrine conditions, i.e. no significant flow, are generally not observed. Furthermore, as the lake is fairly shallow and unstratified, wave action and turbulence caused by storms are significant as sorting mechanisms. This prevents the accumulation of fine materials, except in the eastern part of the lake. Here, a slowdown of the current and increased depth allow finer particles to settle out, and this area could be a potential deposition zone of contaminants.

## Summarized Results (Table 1)

Results for parameters, which are of toxicological interest but found either at very low concentrations or below detection limits, will not be discussed in detail. PBB's and pentachlorophenol were not detected in the 10 samples that were analysed. Chlorobenzenes were either below, or at the most very close to detection limits. Only 3 of the 10 samples gave positive results. Hexachlorobenzene (HCB) was much more common and concentrations ranged up to 13 ng/g.

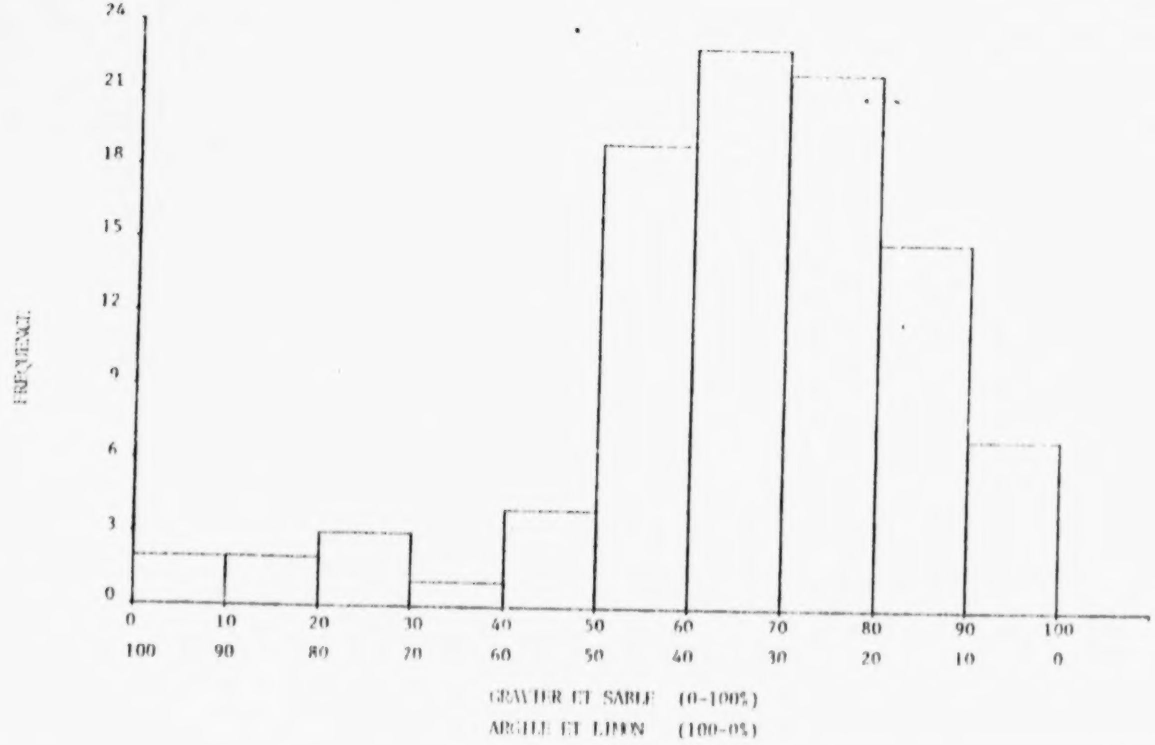

FIGURE 2 HISTOGRAMME DE FRÉQUENCE DES FRACTIONS D'ARGILE-LIMON (%) ET DE GRAVIER SABLE (%) DES SÉDIMENTS DU LAC SAINT-FRANÇOIS PRÉLEVÉS AUX 98 STATIONS D'ÉCHANTILLONNAGE (1979-81)

TABLE 1 SUMMARY OF ANALYTICAL RESULTS (ng/g)

| Parameter | Median | Range |
|---|---|---|
| Hg | 134 | 0.5 - 1474 |
| PCB | 170 | 1 - 1900 |
| PAH total | 552 | 31 - 1883 |
| Benz(a)anthracene | 53 | 4 - 120 |
| Benz(b)fluoranthene | 130 | 7 - 770 |
| Anthracene & Phenanthrene | 32 | 2 - 960 |
| Fluoranthene | 120 | 11 - 360 |
| Benzo(k)fluoranthene | 46 | 3 - 130 |
| Benzo(ghi)perylene | 70 | 2 - 190 |
| Indeno(1, 2, 3-cd)pyrene | 68 | 2 - 900 |
| DDT(total) | 1.5 | <0.1 - 16.7 |
| Mirex | < 1 | <1.0 - 3.3 |
| Chlorobenzenes: | | |
| di-isomers | < 10 | - |
| tri-isomers | < 1 | <1 - 3 |
| tetra-isomers | < 1 | - |
| penta-isomer | < 1 | <1 - 2 |
| hexa-isomer | 0.6 | <0.1 - 13 |
| Pentachlorophenol | < 2 | - |
| PBB | < 10 | - |

Mirex was found at concentrations up to 3.3 ng/g. This is significant in environmental terms and indicates long range aquatic transport of pollutants, as the only known source of mirex is Lake Ontario. These results then confirm that pollutants originating in Lake Ontario are being transported down the St. Lawrence River into the Quebec Region.

In Table 1, results are also given for the parameters which were found at significant concentrations. These will be treated in more detail, although PAH's will be considered as total only. Three carcinogens are included in the list of PAH's analysed, benz(a)anthracene, benzo(b)fluoranthene and indeno(1, 2, 3-cd)pyrene, which were found at concentrations up to 120, 770 and 900 ng/g, respectively. Unfortunately, the significance of these concentrations in ecotoxicological terms is not known.

## Mercury

Results for mercury are summarized in a frequency histogram (Figure 3). Generally, concentrations up to 100 ng/g can be considered as natural (Sloterdijk et Azzaria, 1981). From the frequency histogram it can be seen that about 40% are below this level. The Ontario Ministry of Environment guideline for open water disposal of dredged sediments is 0.3 mg/kg, or 300 ng/g (Levings, 1982). According to this guideline, about 70% of the stations sampled do not present any significant problems. However, it means that 30% of the stations are contaminated, some of which present quite high concentrations, between 1000 - 1500 ng/g.

As can be seen in Figure 4, mercury concentrations are not distributed throughout the lake in a random fashion. A definite pattern is quite evident, the northern part of the lake being much more contaminated than the southern section. It can also be seen that the most contaminated sediments are found in the Cornwall area.

This spatial pattern cannot be explained by differences in particle size. We have to look at possible point sources. At Cornwall, sediments are highly contaminated with mercury, mainly by effluents of a chlor-alkali plant. Up until the beginning of the seventies, this plant was using a mercury electrode. Although mercury is not being used anymore, the contaminated sediments remained, and these are being carried downstream by the prevailing currents. Wave action and turbulence during storms will cause a re-suspension of the sediments, to be dropped further downstream. With time, there is a steady migration of mercury along the north shore of the lake. The south shore does not seem to receive any significant amounts of mercury, nor is there cross-sectional transport from the north shore to the south shore, as is to be expected from the current pattern.

## Polychlorinated Biphenyls (PCB's)

Polychlorinated biphenyls, a mixture of isomers of various chlorine contents, are synthetic compounds and therefore do not occur naturally in the environment. Yet, because of their versatility and specific properties, industrial applications of these compounds have been widespread, with the resulting comtamination of the environment (Task Force, 1976). Today, uses of PCB's are almost completely prohibited (Anon., 1982), although it is expected that sources will continue to exist, and that the PCB problem will remain with us for a while to come.

Results are summarized as a frequency histogram in Figure 5. The Ontario Ministry of the Environment guideline for open water disposal of dredged sediments is 50 ng/g (Levings, 1982). From the histogram it is evident that most stations, about 80%, are

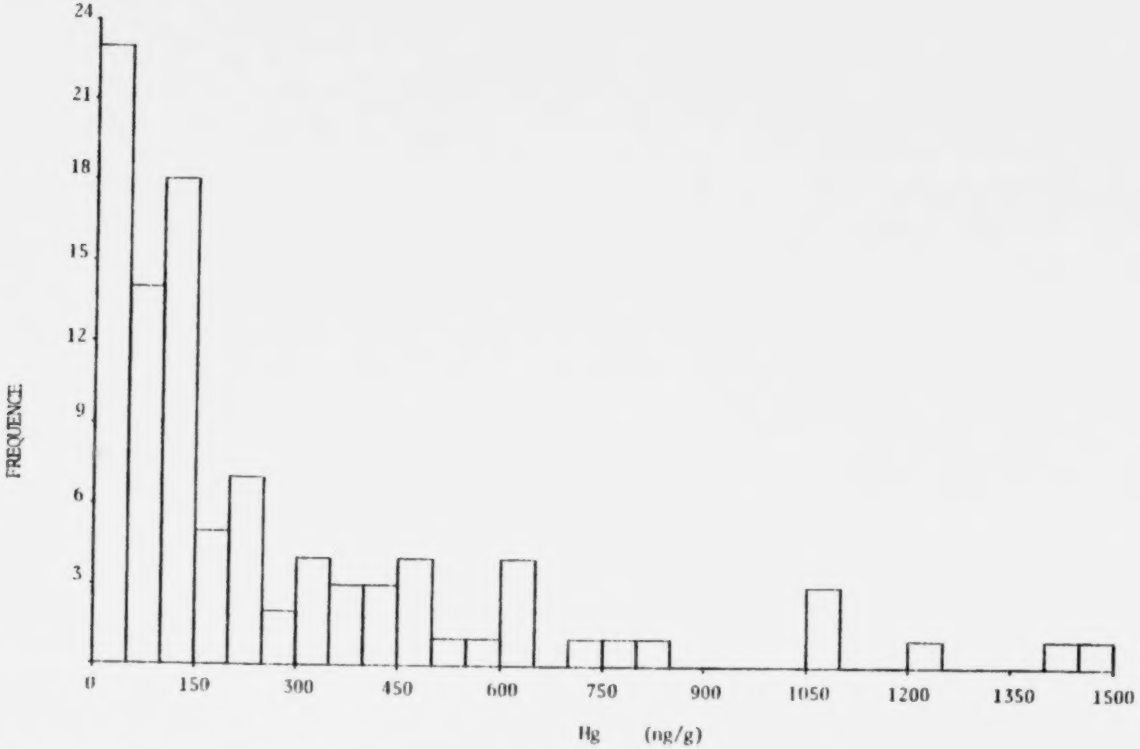

FIGURE 3 HISTOGRAMME DE FRÉQUENCE DES CONCENTRATIONS EN HG (ng/g) DES SÉDIMENTS DU LAC SAINT-FRANÇOIS PRÉLEVÉS AUX 98 STATIONS D'ÉCHANTILLONNAGE (1979-81)

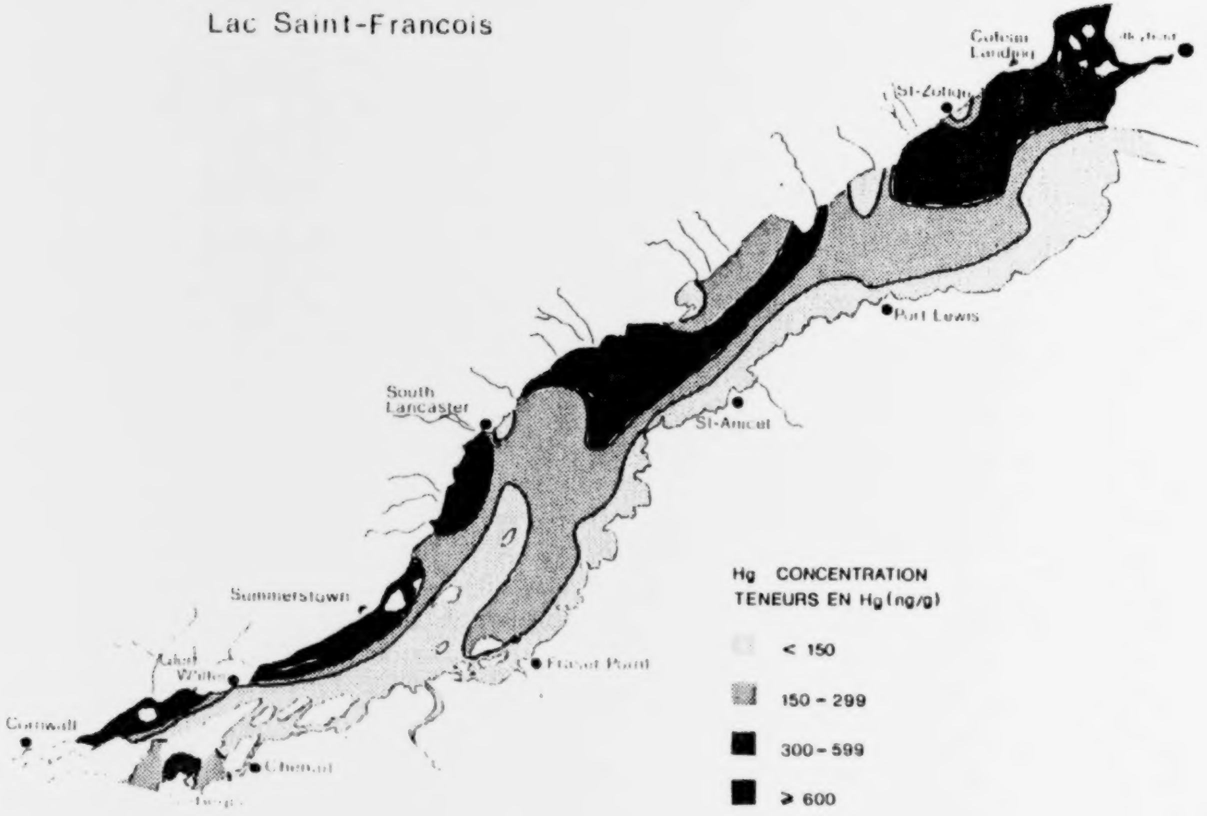
Lac Saint-Francois
Coteau Landing
St-Zotique
Port Lewis
South Lancaster
St-Anicet
Summerstown
Fraser Point
Glen Walter
Cornwall
Chenail
Hg CONCENTRATION
TENEURS EN Hg (ng/g)
< 150
150 - 299
300 - 599
≥ 600

FIGURE 4 SPATIAL DISTRIBUTION OF HG CONCENTRATION IN LAKE ST. FRANCIS SEDIMENTS

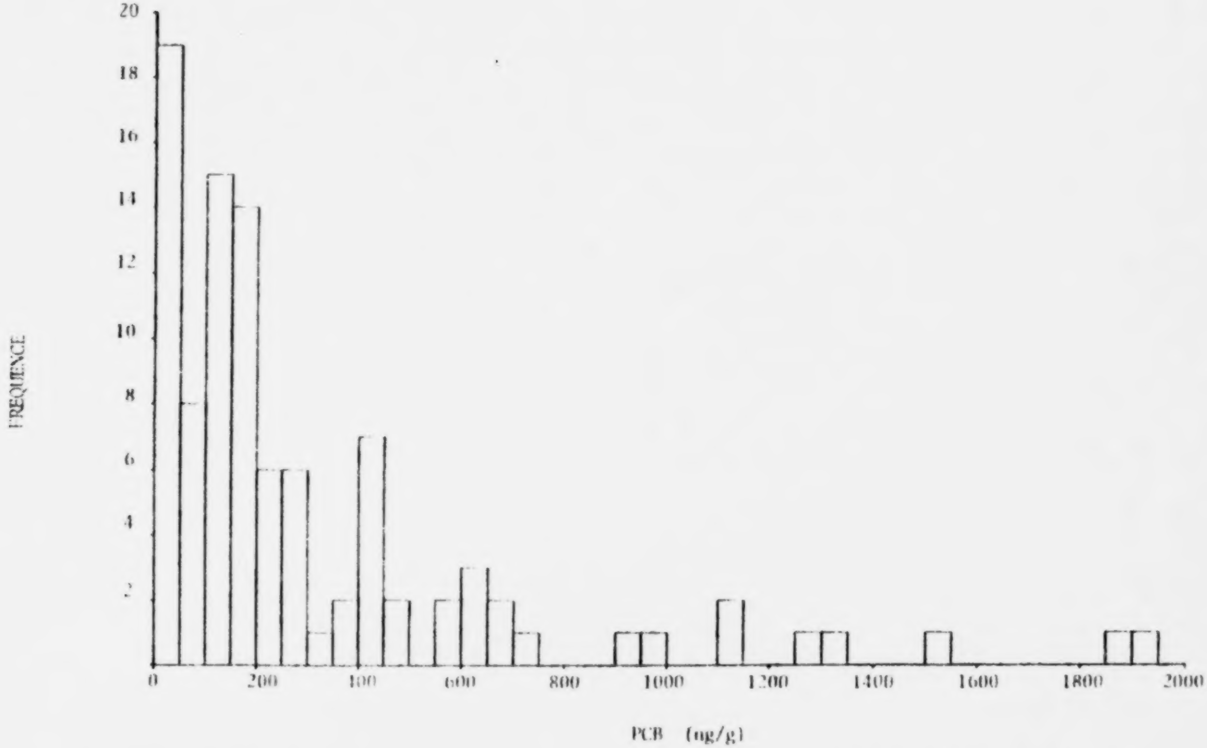

FIGURE 5 HISTOGRAMME DE FRÉQUENCE DES CONCENTRATIONS EN PCB (ng/g) DES SÉDIMENTS DU LAC SAINT-FRANÇOIS PRÉLEVÉS AUX 98 STATIONS D'ÉCHANTILLONNAGE (1979-81)

above this guideline. It can be inferred that they may present problems to the aquatic ecosystem. About 50% of the stations are below 200 ng/g, while about 10% are over 800 ng/g, up to 2000 ng/g.

Spatial distribution is presented in Figure 6. It shows a distinct pattern as well but, in contrast to mercury, we found higher concentrations along the south shore. The highest concentrations are found at the entrance of the lake, along the south shore, and at a few places which seem to be particular deposition zones. This is very evident at the east-end of the lake, already identified as a deposition area based on depth and current velocity.

Particle size or organic carbon contents cannot explain this pattern. There seems to be a source upstream from the lake. It is believed that the New York tributaries of the international section of the St. Lawrence River contribute significant amounts of PCB's to the system. It has been reported that at least one tributary, the Grasse River, is highly contamined with PCB's (Chan, 1980). Thus we have here an example of transboundary pollution, which is brought to light by a sediment survey.

### Polyaromatic Hydrocarbons (PAH's)

Seven different types of PAH'S were analysed (Table 1), of which three are known carcinogens, benz(a)anthracene, benzo(b)fluoranthene and indeno(1, 2, 3-cd)pyrene.

To simplify this presentation, only total PAH's will be discussed. Concentrations for the individual compounds have been presented previously in Table 1.

The spatial distribution pattern is shown in Figure 7. It is apparent that there is a similar pattern as that observed for PCB's: the south shore is generally more contaminated than the north shore. Concentrations are high near the entrance of the lake, especially in the southern section, therefore it seems that New York tributaries are most likely significant sources of PAH's. There is, however, a pocket of high concentrations near the north shore, just downstream from Cornwall. The most likely explanation seems to be the presence of a sewage treatment plant, which has an outlet pipe (diffuser type) just upstream from Pilon Island.

## CONCLUSIONS AND RECOMMENDATIONS

The distribution pattern for PCB's and PAH's indicate high concentrations along the south shore, while mercury concentrations were high along the north shore. It is concluded from this that Lake Ontario may have less an impact on the Quebec part of the St. Lawrence River than was previously suspected. Local sources such as Cornwall and the New York tributaries of the international section of the St. Lawrence are probably responsible for the observed distribution patterns.

However, the fact that mirex was found in significant quantities and that PCB concentrations are high throughout the whole lake, indicates that Lake Ontario remains a significant source of organic contaminants.

Particle size distribution suggests that Lake St. Francis is governed more by fluvial than lacustrine conditions, sand being the predominant fraction at most stations. This then indicates that the lake does not act so much as a contaminant sink, but that most

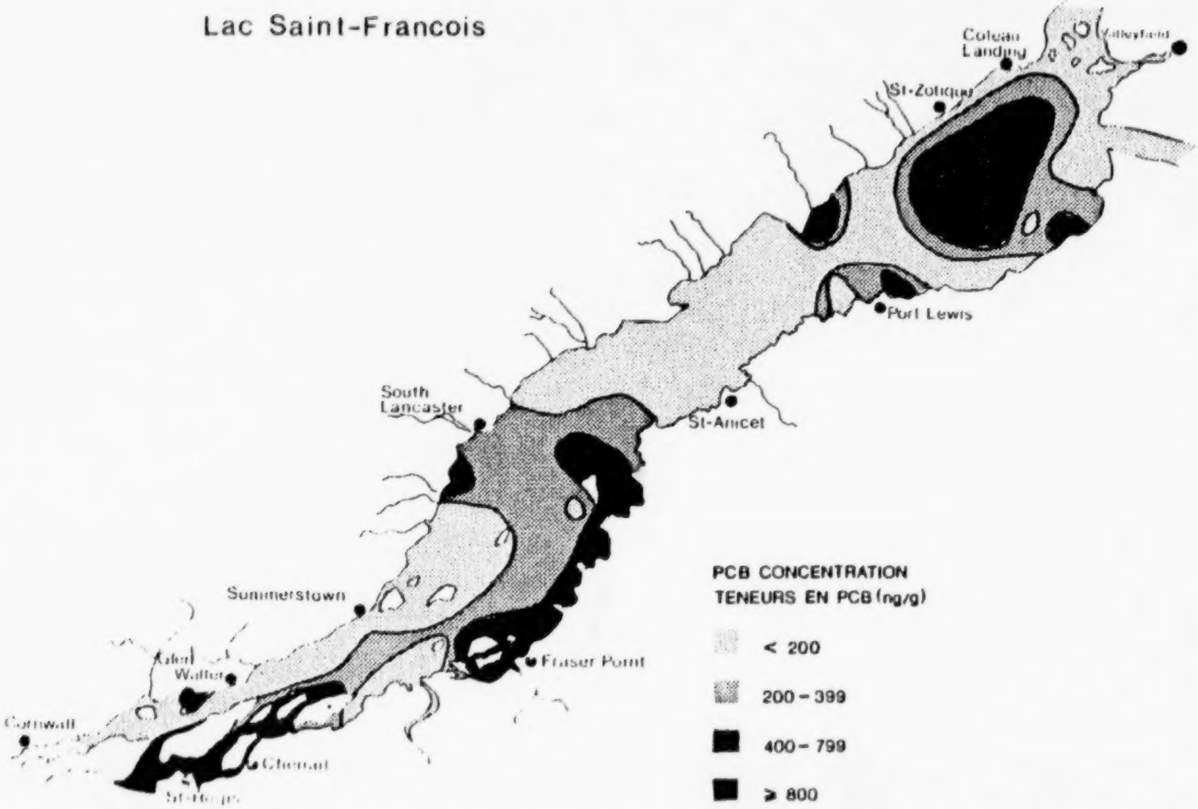
Lac Saint-Francois
Coteau Landing
Valleyfield
St-Zotique
Port Lewis
South Lancaster
St-Anicet
Summerstown
Fraser Point
Glen Walter
Cornwall
Chenail
St-Regis
PCB CONCENTRATION
TENEURS EN PCB (ng/g)
< 200
200 - 399
400 - 799
≥ 800

FIGURE 6 SPATIAL DISTRIBUTION OF PCB CONCENTRATION IN LAKE ST. FRANCIS SEDIMENTS

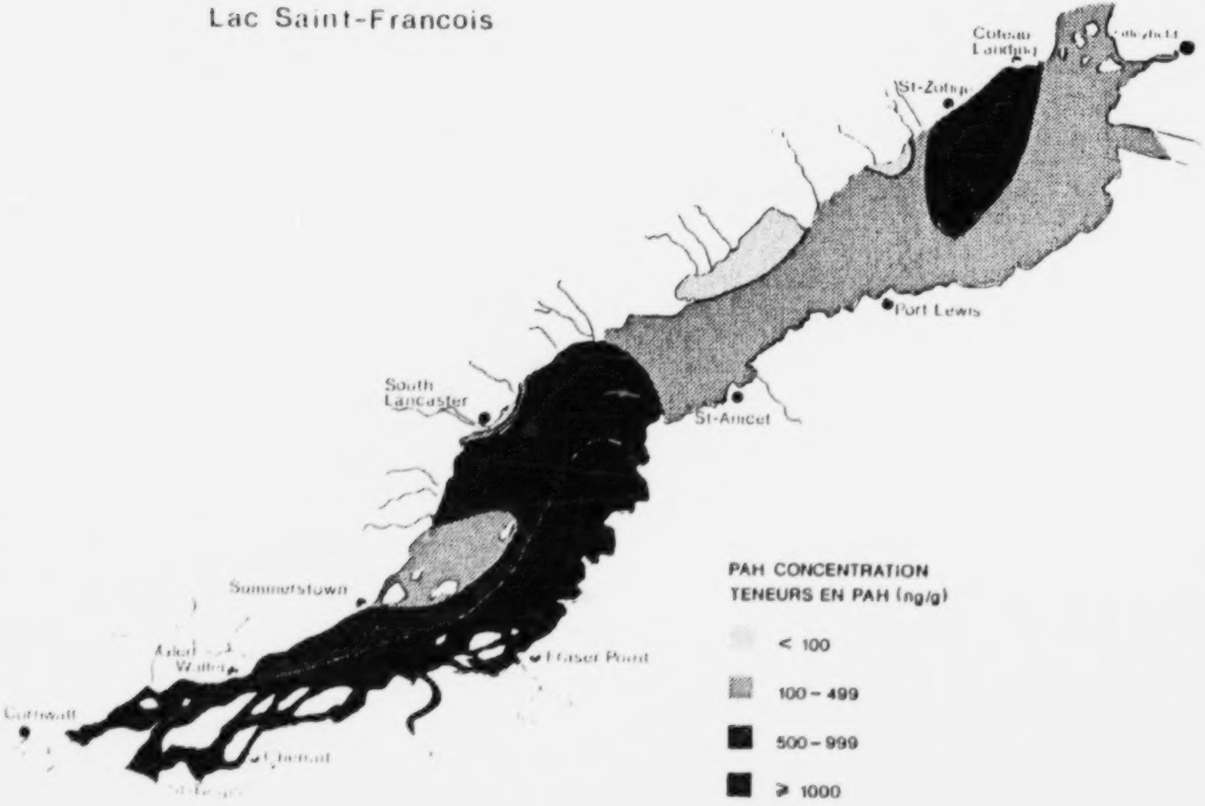
Lac Saint-Francois
Coteau Landing
St-Zotique
Port Lewis
South Lancaster
St-Anicet
Summerstown
Fraser Point
Cornwall
PAH CONCENTRATION
TENEURS EN PAH (ng/g)
< 100
100 – 499
500 – 999
≥ 1000

FIGURE 7 SPATIAL DISTRIBUTION OF PAH CONCENTRATION IN LAKE ST. FRANCIS SEDIMENTS

toxic substances entering the lake from upstream move through the system and out towards the Montreal area.

In closing, it seems appropriate to say a few words on biomonitoring of toxic substances in the environment. This paper has dealt with what might be called "Ambient Level Monitoring", which tells us how much of a given substance is present in a particular environment. However, it does not qualify this information nor determine whether there is a significant impact on the ecosystem.

Therefore, there is an urgent need to develop the tools and methods to study toxic effects or stress factors in the natural environment. As is apparent from some of the papers given at this workshop, bioassays are now carried out using very sensitive and refined techniques, but they are mainly limited to particular toxic chemicals or industrial effluents. These studies are important, and they do provide much information, but it is still very difficult to predict the effects of toxic chemicals that are being found in a particular ecosystem. Ecotoxicology is a popular concept today, yet if we do not address real ecosystems in the field, and not only in the laboratory, then the term ecotoxicology loses its true meaning.

## ACKNOWLEDGEMENTS

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# EFFECT OF THE SOLVENT CARRIER DOWANOL ON SOME GROWTH PARAMETERS OF THE AQUATIC ANGIOSPERM LEMNA MINOR L.

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WEINBERGER, P. and P.-Y. Caux. 1985. Effect of the solvent carrier Dowanol on some growth parameters of the aquatic angiosperm Lemna minor L. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 265-286.

Dowanol TPM (tripropylene glycol methyl ether), is currently in use as an adjuvant in fenitrothion spray formulations. A toxicity rating of this adjuvant has been made using a sensitive non-target aquatic angiosperm, Lemna minor L., as the test organism. Four bioassays were utilized including effects on growth (biomass), photosynthetic disfunction, changes in adenylate charge and change in the electric potential of the bathing media.

A range of concentrations from 0-500 µg/ml Dowanol showed no biotoxic effects in any of the bioassays used. AT 965 µg/ml of Dowanol, fluorometry studies indicated that the compound had a specific deleterious effect on photosynthetic activity. This could be discerned by the fifth day post treatment and by the ninth day, a near 50% reduction of fluorescence was evidenced indicating severe photosynthetic disfunction. This effect was carried over to subsequent daughter generations and consequently, had a deleterious effect on overall metabolism. Dowanol at 965 µg/ml accumulated early (24 hrs) in the treatment period to a level of 22 µg/10 fronds after which depuration was evidenced.

Xenobiotics entering the environment display particular behaviors depending on their physical and chemical properties. The octanol/water partition coefficient for Dowanol was 0.334, thus its bioaccumulation in plant tissue was unexpected. Solvent effects on ATP synthesis and total chlorophyll content in Lemna were also investigated, together with insights into long term effects on chronic stressors.

WEINBERGER, P. and P.-Y. Caux. 1985. Effect of the solvent carrier Dowanol on some growth parameters of the aquatic angiosperm Lemna minor L. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 265-286.

Le Dowanol, ou TPM (tripropylène glycol méthyle éther) est actuellement utilisé comme adjuvant dans des formules de pulvérisation de fénitrothion. On a procédé à l'évaluation de la toxicité de cet adjuvant au moyen d'un angiosperme aquatique sensible non cible, Lemna minor L., comme organisme test. Quatre bio-essais ont été utilisés, soit les effets sur la croissance (biomasse), les troubles de la photosynthèse, les modifications de la charge d'adénylate et le changement de potentiel électrique des milieux aquatiques.

À des concentrations comprises entre 1 et 500 µg/ml, le Dowanol n'a présenté aucun effet biotoxique au cours de nos dosages biologiques. À une concentration de 956 µg/ml de Dowanol, des études de fluorométrie ont montré que le produit avait un effet négatif

spécifique sur la photosynthèse. Cet effet a pu être observé au cinquième jour après traitement; au neuvième jour, une réduction de près de 50% de la fluorescence a été notée, indiquant un trouble prononcé de la photosynthèse. Cet effet s'est transmis aux générations suivantes et par conséquent a eu une action nocive sur le métabolisme en général. Le Dowanol à une dose de 965 µg/ml s'est accumulé dès le début (24 heures) de la période de traitement jusqu'à un niveau de 22 µg/10 frondes; après cette période, on a observé une diminution.

Les xénobiotiques qui pénètrent l'environnement présentent des comportements particuliers en fonction de leurs propriétés chimiques et physiques. Le rapport de composition octanol/eau pour le Dowanol est de 0,334; sa bio-accumulation dans le tissu végétal n'était donc pas prévue. Les effets du solvant sur la synthèse de l'ATP et du contenu total en chlorophylle de Lemna minor ont été également étudiés, avec un aperçu des effets à long terme sur les facteurs de stress chroniques.

# INTRODUCTION

The increase in the worldwide population has brought a concomitant heavy dependence on such products as detergents paints, paint thinners and a range of other household products. The environment is regularly exposed to the emulsifying agents and solvents contained in these products and is therefore subjected to the potential risk that exist for every new chemical synthesized, or new adjuvant in chemical formulations.

Adjuvants contribute significantly to nearly all commercial herbicide/pesticide formulations and are often present at approximately the same concentration as that of the herbicide (Hodgson and Maryland, 1982). The adjuvants are surface active agents or surfactants, with a wide range of applications including wetting agents, detergents emulsifiers, spreading agents, dispersants, foam reducers, solubilizers and penetrants. The type and amount of surfactant used is critical to its subsequent behavior in the environment. As the exact concentration of the components in herbicide/pesticide chemical formulations is not revealed by many of the manufacturers, surfactants have often been used carelessly and in too high a concentration, that is, at concentrations greater than those required for their maximum efficiency as surface tension reducers.

Pesticides are largely applied as water emulsions made from emulsifiable concentrates (Gremlyn 1978). A pesticide formulation comprises the pesticide along with an emulsifier and a solvent or carrier-like substance such as acetone, cyclohexanone, Cyclosol (a fuel oil) and Dowanol (a glycol ether). The use of organic solvents in pesticide formulations is often necessary due to the low water solubility of many pesticides. Their use has however generated potential ecotoxicity to a wide gamut of aquatic organisms (Abel 1974; Berry and Brammer, 1977 and Bode et al. 1978) and terrestrial organisms (Devlin et al., 1979 and William and Goldman, 1981). As these are non-target biota the importance of evaluating carrier or solvent toxicity must be stressed.

The pesticide fenitrothion (0,0-dimethyl 0-(4-nitro-m-tolyl) phosphorothioate), has been used extensively in Canadian forests for the control of lepidopterous defoliators. It is only recently that the toxicity of the components in the formulation have been questioned. The Spitzer report* 1982 has suggested that the use of one component of the fenitrothion formulation, Aerotex, be banned. It is a deleterious naphthalene containing solvent. The Spitzer committee is currently investigating the adjunct Nonylphenol which is now subject to removal from the formulation due to its hazardous environmental side effects. Manufacturers have for too long designated solvent and detergent adjuncts to be chemically "inert" and governments have not required proper toxicological assessments before granting registration. Emphasis has been on the acute stressors having immediate side effects rather than on the chronic stressors having long-term effects. This concern has been addressed in the present study.

Lemna minor L., an aquatic surface dwelling angiosperm, was chosen as the test non-target organism due to its rapid growth, its structural simplicity and its small size making handling and extensive culturing comparatively easy. Its vegetative mode of reproduction reduces genetic variability by the use of single clone inocula in which daughter generations can be identified (Hillman, 1961). Further, it is appropriate to use these organisms due to their importance in aquatic food webs. They are sensitive to metals and other pollutants (Hillman, 1959) and have been studied in a wide array of investigations concerning all areas of plant metabolism (Hillman 1969 1976). Lemna was first used as an indicator species by Nasu and Kugimoto (1981) who found it to be an ideal for this purpose, providing all environmental parameters were controlled. As Lemna dwells on the surface of rivers and lakes, it is a good indicator species for monitoring effects at this interface at which surfactants partition.

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* The Spitzer first report, New Brunswick Task Force

The phasing-out of the carrier solvent Aerotex has resulted in the phasing-in of a new solvent namely that of Dowanol TPM (tripropylene glycol methyl ether). Dowanol is presently used in the fenitrothion formulation as a co-solvent, along with the surfactant Atlox 3409F which is used as an emulsifier.

The tank mix constituents of the formulation includes fenitrothion, Dowanol TPM, Atlox 3409F and water in a w/v ratio of 14.5:1.5:1.5:82.5, respectively. Field concentrations of fenitrothion are 413 g/hectare or 4.13 µg/ml. Field relevant concentrations of Dowanol are 0.43 µg/ml. Its structure is illustrated in Appendix 1.

In this investigation, several bioassays including colony growth and development, biomass, photosynthetic disfunction, chlorophyll content and total ATP were used as determinants of toxicity of the water-soluble spray adjunct Dowanol TPM on the aquatic angiosperm Lemna minor L.

## Materials and Methods

### Test Organism

The test organism used was the aquatic angiosperm Lemna minor L. commonly known as "duckweed". Lemna minor (Lemnaceae family) was obtained from local ponds in the Ottawa region.

### Chemicals

Dowanol TPM (tripropylene glycol methyl ether) was donated by Dow Chemical Co., New Jersey. Firefly lantern freeze dried extracts were obtained from the Sigma Chemical Company (50 mg of extract in magnesium arsenate buffer) and stored at -20°C. Adenosine 5 triphosphate (FF ATP) was also obtained from the Sigma Chemical Co. and was stored at -20°C either intact or as a 10 µg/ml stock solution for ATP standards. Potassium and magnesium stock solutions were obtained from the Fisher Scientific Co. and kept at room temperature.

### Instrumentation

A Plant Productivity Fluorometer (model SF-20) (Branker Res. Ltd., Ottawa, Canada), was used at a maximum light intensity of $10^4$ ergs/cm$^2$/sec and set at a light exposure of 50 sec. and a gain of 0.8. The L.E.D. emitter emits relatively monochromatic light with a peak wavelength of 670 nm at a current of 50 mA. The fluorometer was used in conjunction with a Keithley Instrument Series 370 chart recorder (signal input 10 vdc, chart speed 12 cm/min). The fluorometer monitored photosynthetic activity. A Beckmann L.S. 3133P liquid scintillation counter was set at an open window of 0-1000 and a gain of 52 and was used to monitor ATP content. A conductivity meter (Back Simpson Ltd. Model No. CMD2 11R116N32) served to monitor changes in the electropotential of the bathing media. A Unicam SP 1800 Ultraviolet Spectrophotometer set at a wavelength of 652 nm monitored chlorophyll content. A Varian AA-175 Series Atomic Spectrophotometer was used to measure specific ion leakage from Lemna plants. Two separate lamps were used for potassium and magnesium analyses. Specific element conditions are given below:

| Element | Current (mA.) | Slit Width (nm) | Wavelength (nm) | Flame* |
|---|---|---|---|---|
| $K^+$ | 5 | 0.2 | 766.5 | A-A |
| $Mg^{++}$ | 3 | 0.5 | 285.2 | A-A |

* The Flame used as an Air-Acetylene flame.

Gas chromatographic analyses were performed on a Hewlett Packard Model 5880 gas chromatograph equipped with an FID detector. A 1.2 m 2 mm i.d. glass column packed with 10% Carbowax 20M on 80/100 mesh was maintained at 150°C with 30 mg/min carrier ($N_2$) flow. The injection port and detector were kept at 150°C and 300°C respectively.

## Growth Conditions

Plants were grown in 500 ml Erlenmeyer flasks in 200 ml of Bowker's media (Bowker, 1980) at 25°C ± 1°C, under a constant illumination of 12 000 lux produced by a bank of Westinghouse cool white fluorescent lights in a Sherer incubator (Model CEL 255-6). Plants were obtained from an axenic stock culture following the sterile growth procedures of Bowker *et al.* (1980). Sterile conditions were maintained throughout to exclude any other organic growth which might interfere with viability or development of *Lemna minor*, or alter the chemical characteristics of the test chemical. Clonal populations were developed by vegetative multiplication of a single frond inoculum (R-3 colony) (Hillman, 1961).

## Growth Studies

Preliminary screening: Three plants or ten fronds were used to inoculate each flask. Plants grown in media alone served as controls. Fronds were treated with Dowanol at concentrations of 96.5, 482.5 and 965 µg/ml. Numerical counts of the fronds in each flask were taken on alternate days for a period of ten days after which the fresh and dry weights of the plants in each flask were recorded. In addition, fluorometry and conductivity measurements were also taken (details below).

Equilibrium distribution studies: Six mother fronds with no apparent directional growth neither left nor right side were inoculated into six separate flasks. Following equilibrium distribution the fronds in each flask were harvested and sorted into either a left side, (undergoing the left hand hemicycle of the growth cycle) or right hand side, (undergoing the right hand hemicycle to the growth cycle).

## Generation Studies

In this study, a total of 28 flasks were inoculated with right hand side mother fronds at stage R-3* (R-3 being analogous to stage L-3 in Datko et al., 1980). Two generation studies were undertaken. The first, simply to follow the mother generation and the second, to follow the daughter generation.

A) Mother generation: Twelve flasks were inoculated with one R-3 colony. Of these, eight were control sets and four treatment sets (965 µg/ml Dowanol). In each set the mother frond was marked with a tiny spot of white liquid paper fluid (Liquid Paper Co., Toronto, Ont.) at the distal end of the frond away from the meristematic centers. Four of the controls were left unmarked to test marking effects. The advantage of using liquid paper was that it is fast drying and insoluble in water enabling quick and easy marking while maintaining sterility and minimizing handling of the plant tissue.

As daughters emerged from the right hand pocket and reached the R-5 stage, they were excised from the mother fronds, (a slight pull was sufficient) and were tested fluorometrically. Mother fronds were returned to the bathing media till the next right handed daughter emerged. Fronds arising from the left pocket were discarded. The mother generation is illustrated in Figure 1.

B) Daughter generation: Three R-3 mother colonies marked with a tiny white spot were inoculated into each of the remaining sixteen flasks. Of these, eight provided treated sets containing 965 µg/ml Dowanol and eight untreated controls. As the first daughters became prominent and reached the R-5 stage, they were marked with a tiny blue spot and one plant out of each of the four treated and four control flasks were harvested. On these, fluorometric determinations were obtained. The next right handed daughter of the blue marked mother, was spotted yellow. Right pocket daughter fronds following this previous sequence, were marked in red, orange, and finally in white, giving a total of six right handed generations. Fronds arising from the left pocket were again discarded along with any other previously marked fronds of earlier generations. The daughter generation is illustrated in Figure 2.

## Photosynthetic Disfunction

Plant fluorometry: This technique as modified by Moody et al. (1981), has proven to be a rapid and efficient method to assess the relative toxicity of environmental pollutants. On sampling days, an R-3 or L-3 plant from each flask was harvested while maintaining sterile techniques. The fronds were placed on plastic sheets specially conceived so that the fluorometer probe was centered directly on top of the Lemna frond. A three minute dark adaptation period was allowed, to permit the electrons of the photosynthetic apparatus to regain their ground state. Exact timing was required to obtain reproducible results. Previous studies by Krause (1973) showed that the size of the initial fluorescence peak was directly proportional to the length of the preceeding dark period.

Thirty fronds were inoculated in each of eight flasks, four were control sets and four Dowanol (965 µg/ml) treated sets. Following frond doubling, half of the fronds from each

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* The reasoning for choosing R-3 will be clarified later.

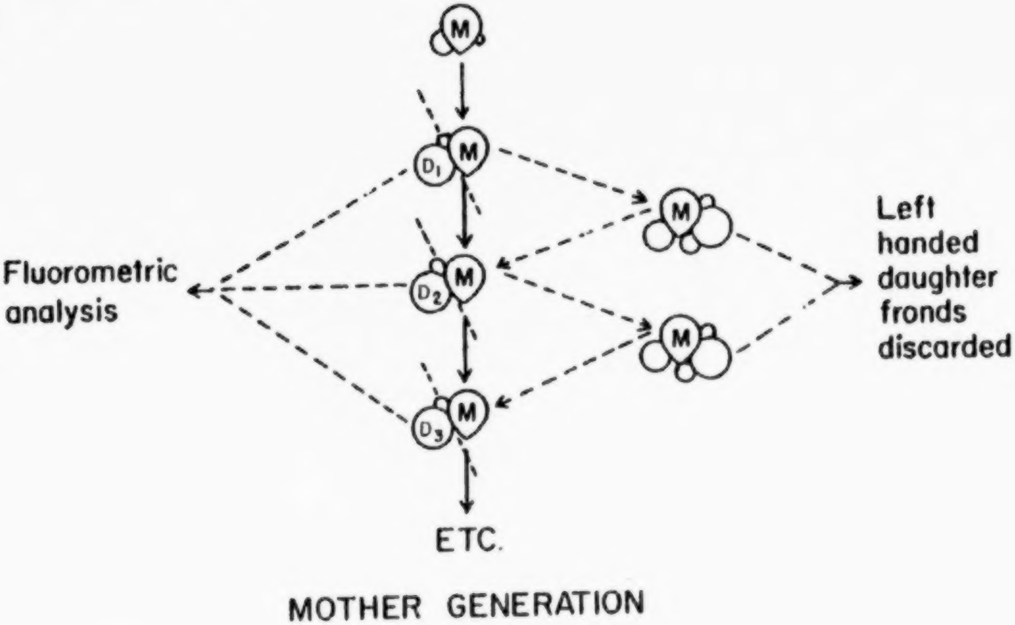
M
D1
M
D2
M
D3
M
M
M
Fluorometric analysis
Left handed daughter fronds discarded
ETC.
MOTHER GENERATION

FIGURE 1 FROND EMERGENCE FOR FLUOROMETRIC DETERMINATION ON MOTHER GENERATION

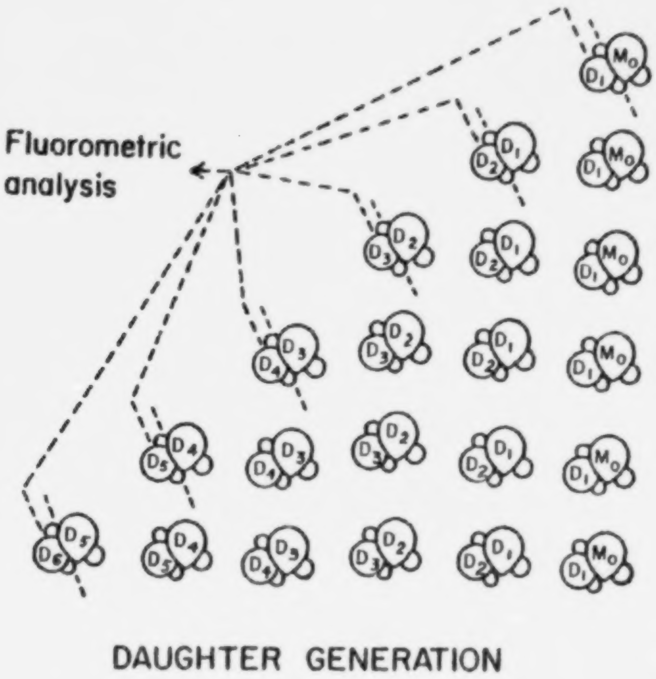
Fluorometric analysis
Mo
D1
D2
D3
D4
D5
D6
DAUGHTER GENERATION

FIGURE 2 FROND EMERGENCE FOR FLUOROMETRIC DETERMINATION ON DAUGHTER GENERATION

of the flasks were harvested and placed in eight new flasks containing the exact growth conditions to which they had previously been subjected. Once again, when doubled in frond number, half the fronds were transferred to a new set of flasks until 36 control and 36 treatment were utilized. From these were obtained the frond number, fluorometer, total chlorophyll, conductivity and ATP data on all sampling days.

Chlorophyll analysis: Lemna minor plants were treated with Dowanol at a concentration of 965 µg/ml. Each flask was inoculated with 60 fronds, 30 of which served for ATP analysis and 30 for chlorophyll analysis. Six flask were inoculated for each of the six time periods corresponding to 6, 12 24 48, 96 and 192 hours. Of each six flask, three served as controls and three as treated sets. After each time period, 30 fronds were harvested and their fresh weights were taken. These were then placed in 5 ml mini vials containing 80% acetone and stored overnight at -20°C. Total chlorophyll content was measured by spectrophotometry. After completion of the analyses, the vials were placed in an oven and the dry weights of the fronds were taken as soon as the vials equilibrated to room temperature.

## Change in Adenylate Charge

The remaining 30 Lemna fronds were harvested and frozen at dry ice temperatures (-40°C) in ethanol (5 ml) and then stored overnight at -20°C. The ethanol fraction was removed, the plants were lyophilized to remove the residual ethanol and they were then exhaustively extracted in (4 ml) Tris buffer at 100°C to obtain ATP extracts which were kept frozen until analysis (Gower, 1981). Deionized distilled water (37.5 ml) was used to rehydrate each firefly lantern freeze dried extract vial and the suspensions were left to stand at room temperature for 1 hr after which they were filtered (Whatman No. 4) and the filtrate incubated in an ice bath for 24 hr (Patterson et al., 1970). Each of the extracted samples (0.5 ml) was mixed for 30 s with firefly extract (1.5 ml). The scintillation counter measured the light emission during the 1 min. oxidation of luciferase. This provided a measurement of the ATP content of the fronds. Final ATP content was determined from a standard calibration curve.

## Conductivity and Specific Leakage

From each flask approximately 5 ml of media was decanted and put into separately labelled mini vials. The media in these was used to measure changes in the electric potential of the bathing solution along with potassium and magnesium specific ion leakage.

Conductivity: The conductivity of the bathing solutions of control and treated sets was measured daily (0-28 d) immediately following fluorometric analyses.

Specific ion leakage: The same 5 ml mini vials which had previously been used to measure conductivity were used to measure potassium and magnesium concentrations in the bathing media.

After the spectrophotometer had been set for the element condition required the machine was zeroed with deionized distilled water and the standards as well as all treated and control sample values were recorded. Final specific ion content was obtained from a calibration curve.

## Accumulation Studies

At time intervals of 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 24, 48, 96 and 240 hr, six flasks of which three were control and three Dowanol (969 µg/ml) treated, each containing populations of Lemna initiated with a 30 frond inoculum at time zero, were taken and their frond content was harvested. These were then separately placed in 15 ml vials containing 5 ml methanol and dismembranated with the Fisher (Model 300) sonicator (35% power for 10 sec.). Following an overnight storage at -20°C, the methanol was blown off with nitrogen gas to give a final volume of 0.5 ml. The extracts were centrifuged in a clinical centrifuge at 500 g for 5 min. The supernatants were then separately transferred to 10 ml vials and the debris pellets were redisolved in 2 ml of methanol, centrifuged and the supernatant transferred, together with the original supernatant to minimize the loss of Dowanol. The combined supernatant extract was reduced to 0.5 ml for GC analysis.

## Depuration

The inoculation procedure was as described, however, the experiment was initiated with 36 flasks and was expected to run for a period of two weeks. After two days, all fronds were transferred into new flasks containing fresh untreated media. Transfers were made every three days to prevent crowding effects. Fluorometric measurements were made every other day.

## RESULTS AND DISCUSSION

Dowanol is infinitely soluble in water and its octanol/water partition coefficient as presently determined by G.C. analysis is 130:334, which is very low. The significance of this is that the compound is expected to be minimally lipophillic and thus not bound to membrane lipids. The molecular connectivity index for Dowanol, which gives an indication of its sorption and bioconcentration properties as well as its biological activity was calculated to be 3.229 in the first order index. This value is relatively low as compared to some of the chlorobiphenyls and DDT which are of the order of 7.5.

The growth habit of the Lemna colony used in this study indicated that the colony produced 67% right handed: left handed daughters. Since most of the colonies were traversing their first two growth cycles it was fairly simple to obtain representative samples of a constant colony type, that is a right handed "R-3" colony for experimentation.

Growth studies in the presence of a range of Dowanol concentrations of Dowanol (Table 1) showed that the two lower concentrations (96.5 and 482.5 µg/ml) did not affect any of the parameters assayed. It was only at a high concentration of 965 µg/ml that significant effects were observed.

In the generation studies, the tiny spot of liquid paper did not affect photosynthetic activity in Lemna. No aberrant photosynthetic effects were detected when following the mother generation when it was subjected to Dowanol at a concentration of 965 µg/ml.

TABLE 1 PRELIMINARY SCREENING OF THE EFFECTS OF DOWANOL ON THE GROWTH OF L. MINOR. DATA ARE GIVEN AS % CONTROL

| Dowanol (µg/ml) | 965 | 482.5 | 96.5 |
|---|---|---|---|
| No. of fronds | 61 a | 81 n.s. b | 104 b |
| Fresh weights | 74 a | 80 n.s. b | 102 b |
| Dry weights | 68 a | 83 n.s. b | 107 b |
| Fluorometry | 55 a | 91 n.s. b | 105 b |
| Conductivity | 107 b | 109 n.s. b | 105 b |

a Significance determined by the student t. test at the .05% level.
b Non-significance observed according to t. test at the .05% level.

When following the daughter generation, a significant difference was observed at day 12 post treatment with Dowanol as is illustrated in Figure 3. According to these results the 7th daughter generation may be affected. This 7th generation would be a right handed $D_1M_3$ or 1st daughter of a 4th mother as the first mother is always designated as $M_0$. Photosynthetic activity is directly related to electron flow at photosystem 2 (PS2) and is measured by the fluorescence emitted from the leaf, this is related to the difference between P and T transients; for more details on the various photosynthetic transients refer to Schreiber *et al.* (1978) and Moody (1981). An effect on daughter generations, as opposed to mother generations would indicate effects on growth metabolism or possible genetic aberrations.

The effect on photosynthetic activity on aggregate generations of *Lemna*, that is all types of fronds, is shown in Figure 4. A significant reduction in photosynthetic activity was observed from days 2-13. Nine days post-treatment 55% reduction was observed. This was followed by a full recovery at day 15. Recovery was maintained thereafter till the end of the experiment on day 28.

The results of the spectrophotometric analysis of the chlorophyll content of the treated and control fronds is illustrated in Figure 5. A 35% decrease 24 hours post-treatment was observed. Analysis of ATP via the luciferin-luciferase scintillation method is illustrated in Figure 6. A similar trend was observed. A 25% decrease 24 hours post-treatment was evidenced.

Effects on media conductivity were however, not apparent during the span of the 28 day experiment, Table 2.

At 965 µg/ml, Dowanol TPM in plant tissue would act as a general depressant. It is conceivable to suggest that the highly hydrophillic Dowanol molecules, which seem not to affect the membrane electropotential may reach the photosynthetic site of the thylakoid membranes and obstruct the Hill reaction, thereby reducing photosynthetic activity. The

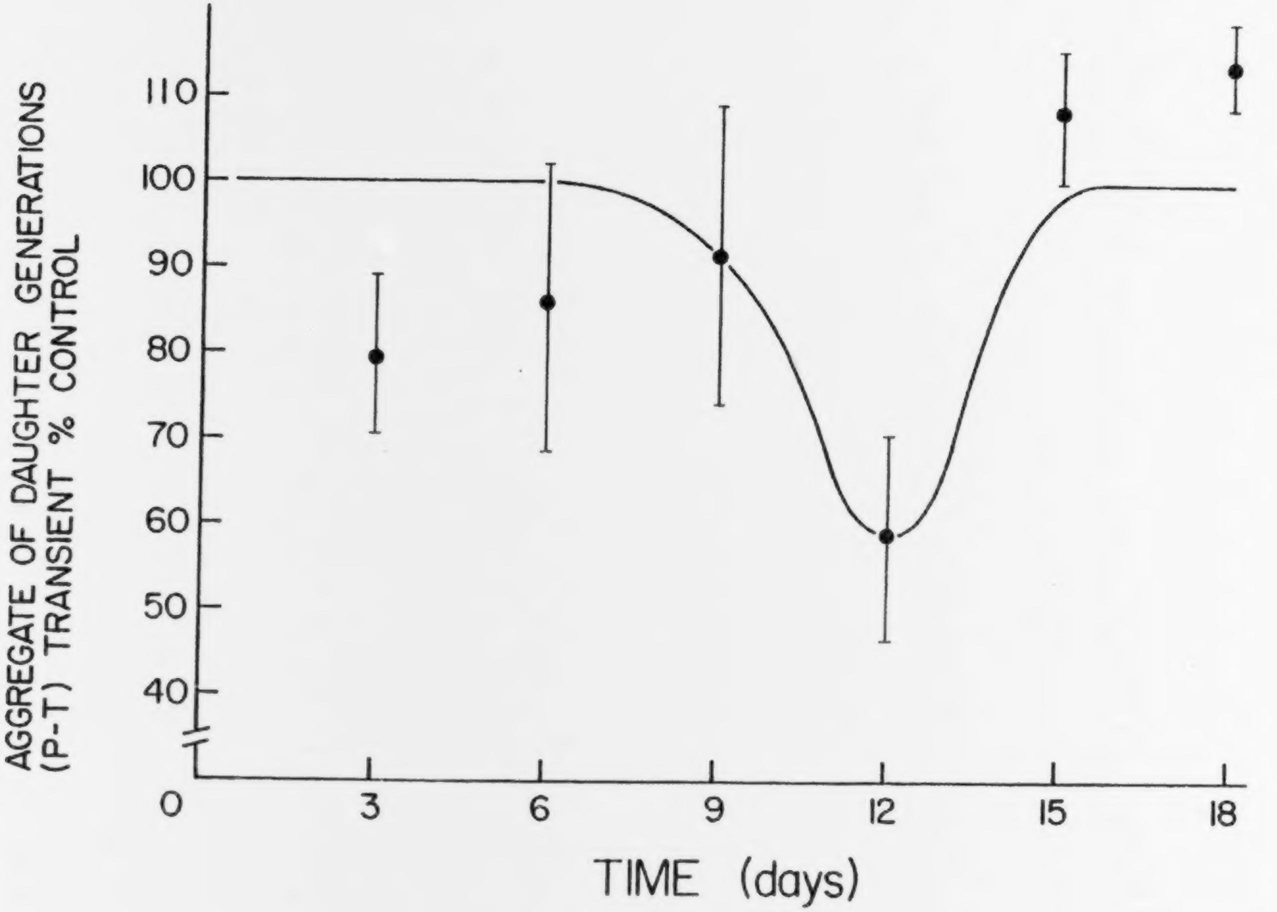

FIGURE 3 AGGREGATE OF DAUGHTER GENERATIONS (P-T) TRANSIENT PHOTOSYNTHETIC EFFECTS

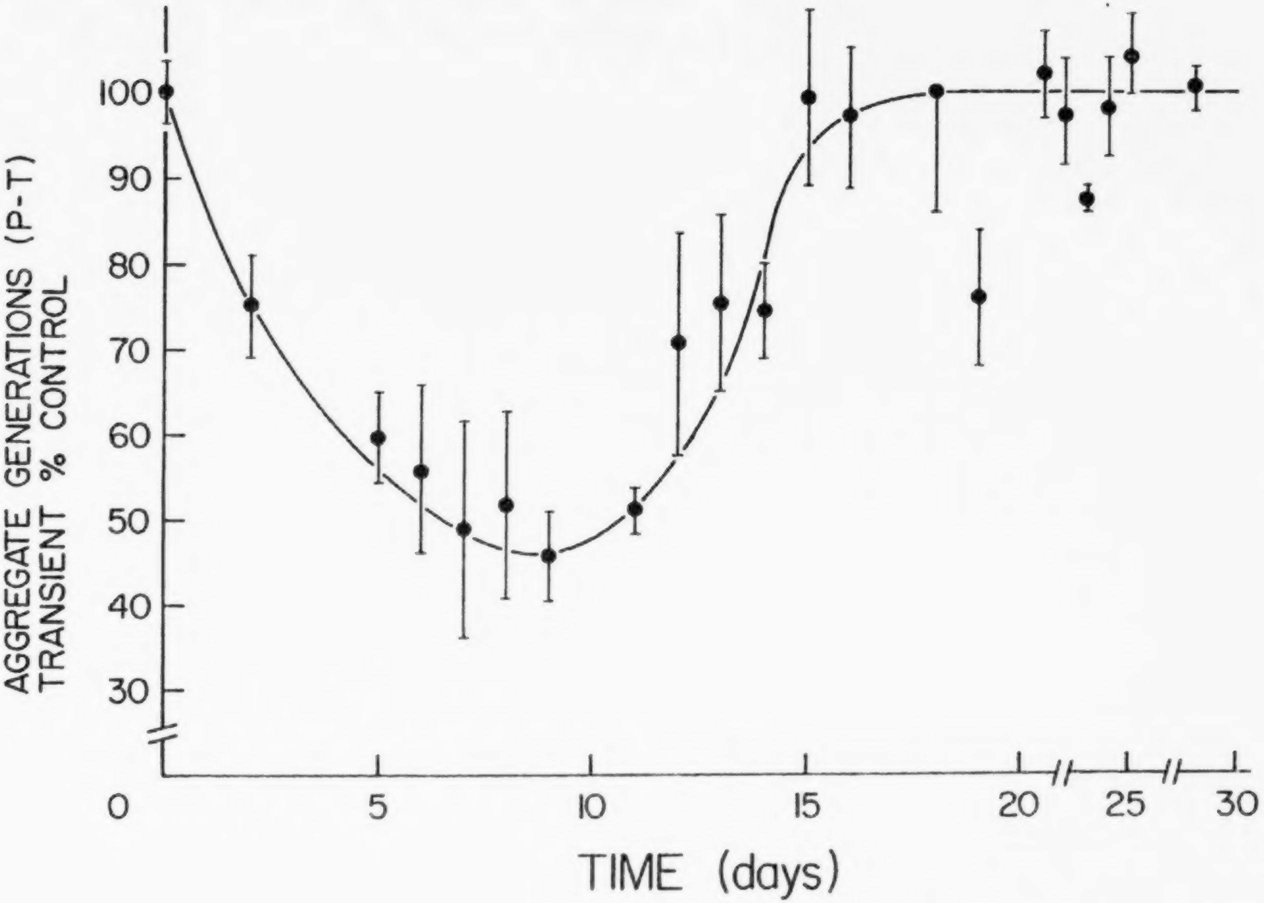

FIGURE 4 AGGREGATE GENERATIONS OF LEMNA MINOR (P-T) TRANSIENT PHOTOSYNTHETIC EFFECTS

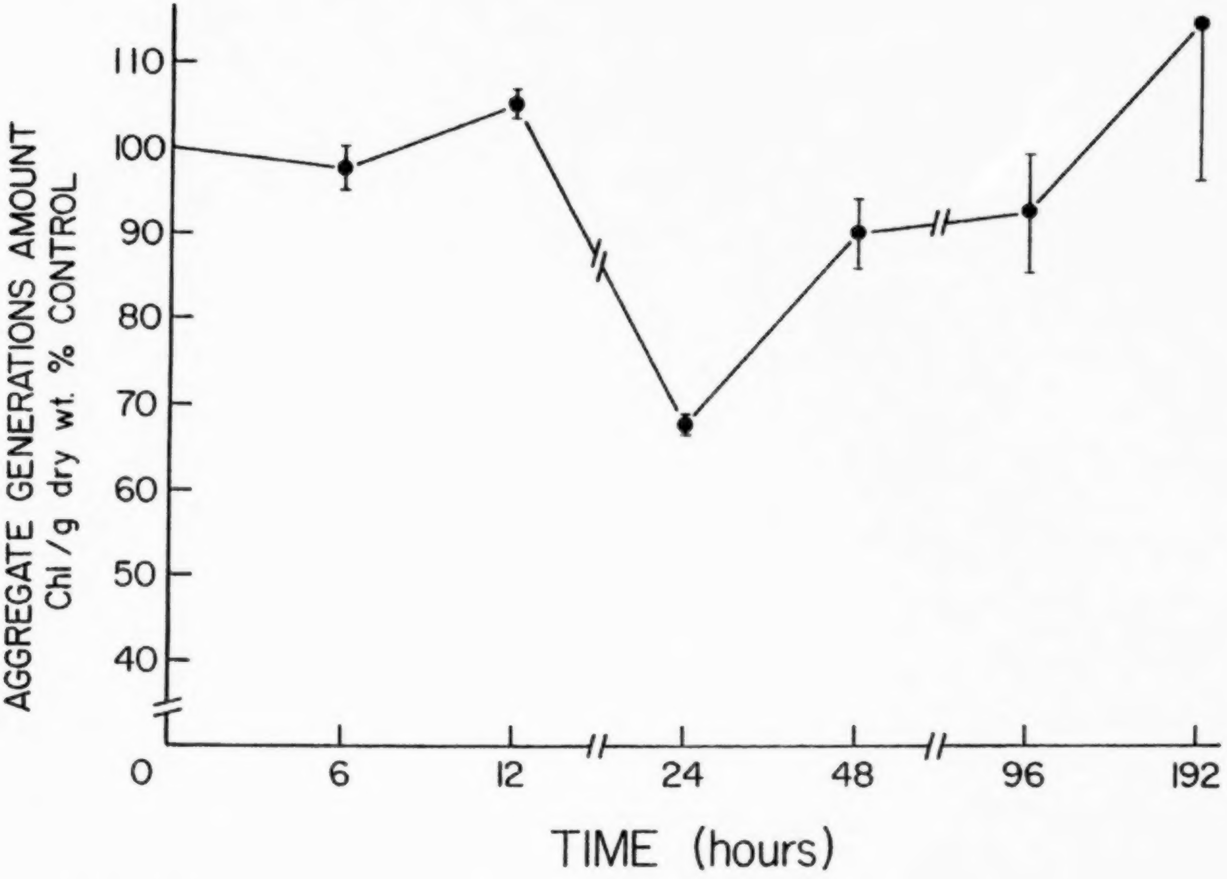

FIGURE 5 CHLOROPHYLL CONTENT IN LEMNA MINOR PLANTS SUBJECTED TO DOWANOL TPM AT A CONCENTRATION OF 965 μ G/ML

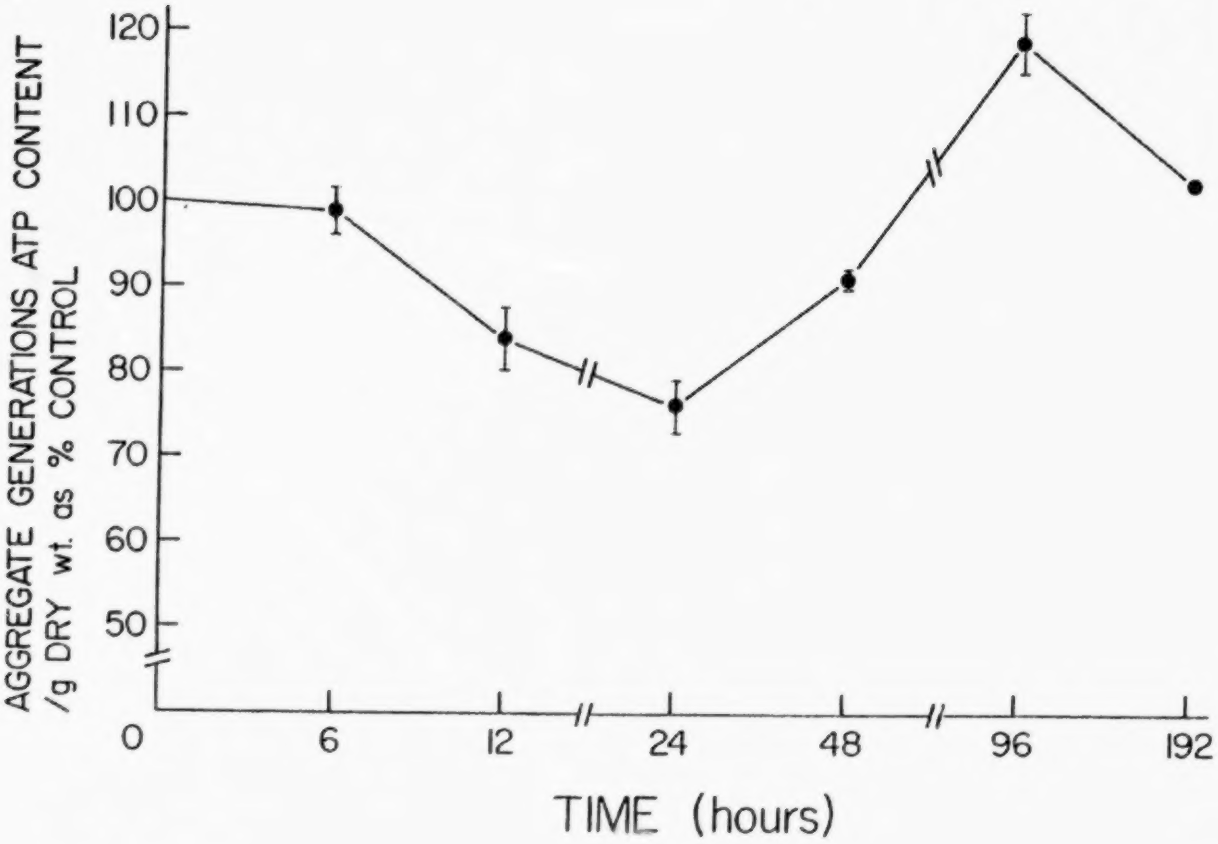

FIGURE 6 ATP CONTENT IN LEMNA MINOR PLANTS SUBJECTED TO DOWANOL TPM AT A CONCENTRATION OF 965 μG/ML

TABLE 2 MEDIA CONDUCTIVITY: NUMBERS IN TABLE REPRESENT THE AVERAGE CONDUCTIVITY OF THE BATHING MEDIA ON A PARTICULAR DAY AS % CONTROL

| Day | % | Day | % | Day | % |
|---|---|---|---|---|---|
| 0 | 97 | 15 | 100 | 23 | 101 |
| 3 | 102 | 16 | 100 | 24 | 98 |
| 6 | 103 | 18 | 100 | 25 | 101 |
| 9 | 104 | 21 | 99 | 28 | 102 |
| 12 | 96 | 22 | 100 | | |

Mean: 100.2

Standard Deviation: 2.225

Variance: 4.597

active site of the molecule, the hydroxyl group, would have to participate in the redox water-splitting reaction, thus invoking an inefficient system and as a consequence, there would be a decrease in photosynthetic activity and overall metabolism such as is indicated by the effects on growth biomass and ATP content noted in this study.

Phytotoxicity via chlorophyll bleaching has recently been well documented (Gerhard and Boger, 1983), and may be due to an apparent peroxidation of membrane bound polyunsaturated fatty acids together with a sequential breakdown of carotenes and chlorophylls, along with further decay of electron transport.

As conductivity was unaffected, further analysis of specific ion leakage was performed by atomic absorption. Figure 7 illustrates the isotherm obtained for the potassium ($K^+$) concentration which seems to indicate that a leakage of the monovalent $K^+$ ion occurred 15 day post-treatment. Figure 8 illustrates the curve obtained for the magnesium ($Mg^{2+}$) concentration. Again, leakage of $Mg^{2+}$ ion into the media occurred around the 15 day post-treatment. There are as yet no plausible explanations for such a delay in ion leakage. A possibility could involve sequential perturbations. Primary acute effects were witnessed early, 24 hours post-treatment, whereas ATP and chlorophyll content were probably reduced via electron flow inhibition and chlorophyll breakdown. Secondary effects were observed to occur 9 days post-treatment, where photosynthetic activity was significantly reduced. Tertiary effects would signal an alteration of the membrane integrity noted 15 days post-treatment, demonstrated by the leakage of electrolytes.

Time dependant accumulation of Dowanol is shown in Figure 9, where an accumulation of approximately 22 μg/10 fronds was obtained. Maximum accumulation occurred at

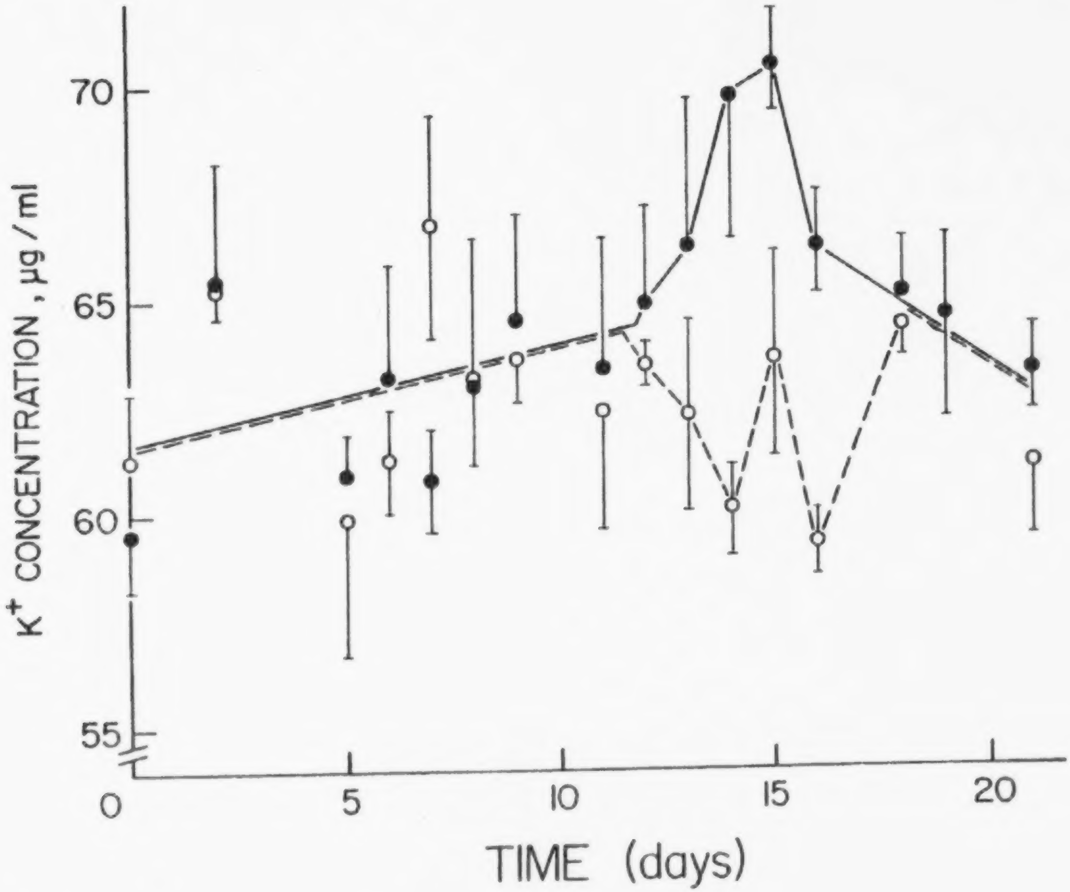

FIGURE 7 SPECIFIC ION LEAKAGE, LEAKAGE OF THE MONOVALENT K+ ION INTO THE BATHING MEDIA BY LEMNA MINOR PLANTS SUBJECTED TO DOWANOL TPM AT A CONCENTRATION OF 965 μG/ML

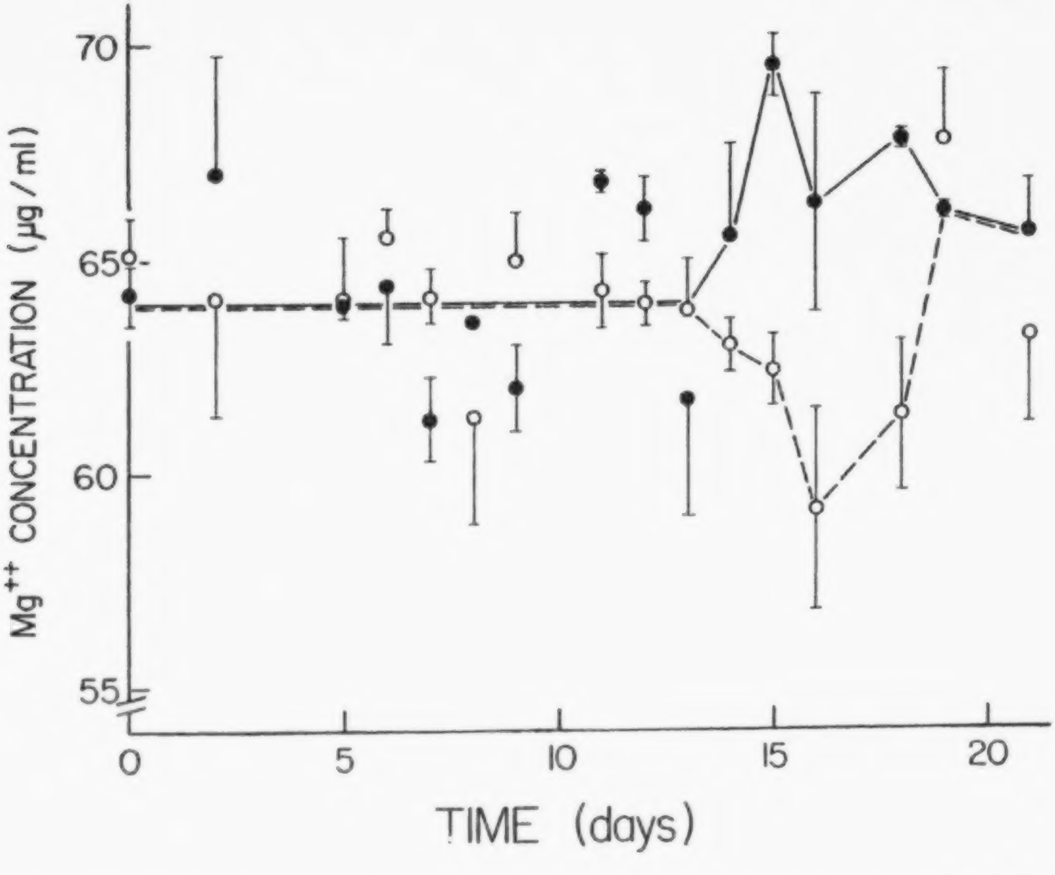

FIGURE 8 SPECIFIC ION LEAKAGE, LEAKAGE OF THE DIVALENT $Mg^{++}$ ION INTO THE BATHING MEDIA BY LEMNA MINOR PLANTS SUBJECTED TO DOWANOL TPM AT A CONCENTRATION OF 965 μ G/ML

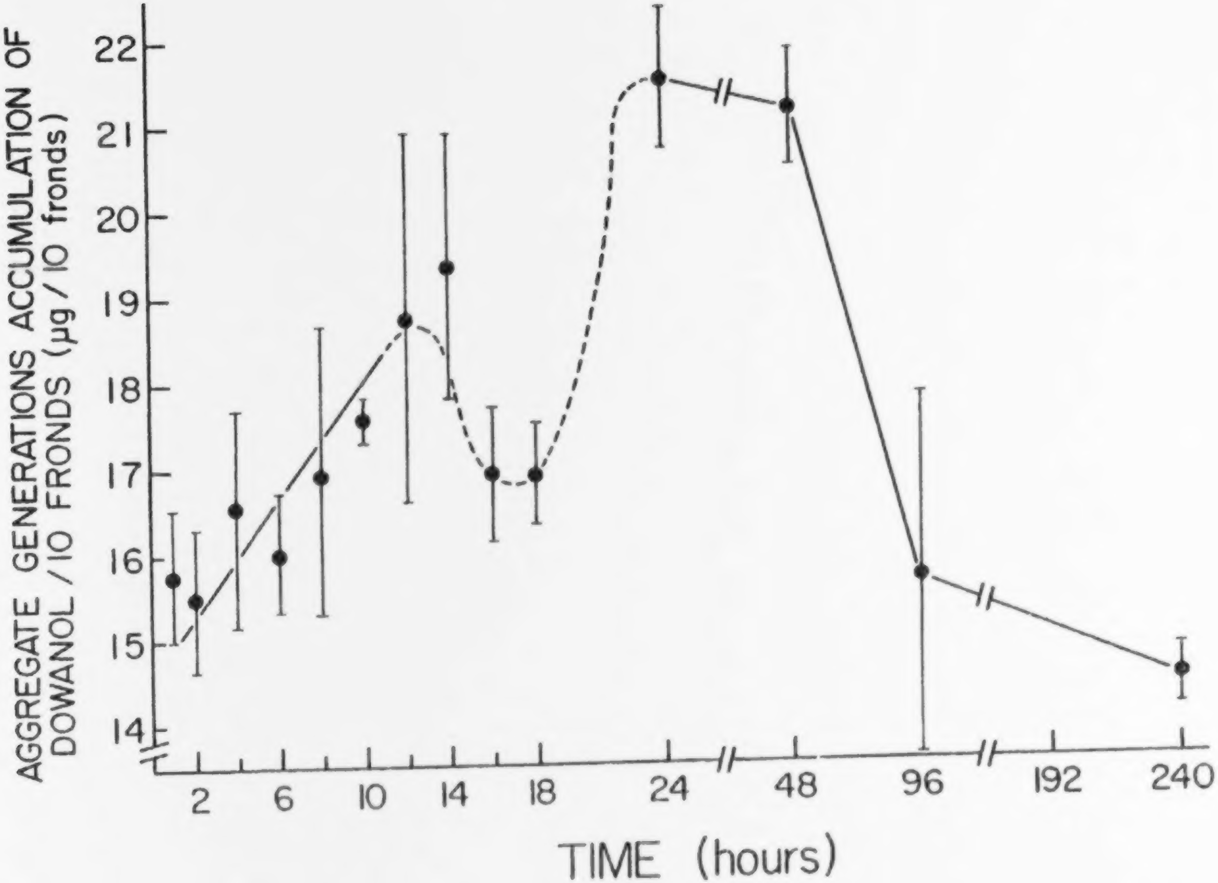

FIGURE 9 ACCUMULATION STUDIES, AGGREGATE GENERATIONS ACCUMULATION OF DOWANOL TPM IN *LEMNA MINOR*

24 hours post-treatment and was maintained until 48 hours whereafter a depuration was observed. This was followed by depuration of all the previously sorbed surfactant.

Accumulation of Dowanol to potentially toxic levels in organisms exposed to the chemical at field relevant concentrations was unexpected. However, in vivo concentrations at 22 μg/10 fronds may be indicative of the toxic threshold level that may have to be accumulated by Lemna in order for phytotoxic symptoms to be observed. It should be noted that after 24 hours treatment concurrent depressant effects were obtained in chlorophyll and ATP content.

The accumulated Dowanol could be rapidly cleared from the Lemna fronds. Figure 10 illustrates the results obtained for a depuration experiment where fronds were transferred to fresh untreated media after exposure to Dowanol for a period of two days. It can clearly seem that a prompt recovery was observed soon after transferral. Such recovery is likely due to an efflux of Dowanol from the frond and root tissues, resulting in concentration levels below the toxic threshold. With continual exposure it took a little over a week for the Lemna to adapt to the chemical stress and begin a recovery phase. A two day exposure was insufficient to obtain maximal detriment to photosynthetic activity. Thus, the length of exposure, prior only to the 9th day maximal inhibition point, will determine the extent of the photosynthetic disfunction.

A final comment deals with the need for proper phytotoxic assessment of every new chemical introduced into our environment. Assessment to non-target species should be provided at a biomolecular and physiological level. Further, persistance of the chemical and its chronic effects should be stressed.

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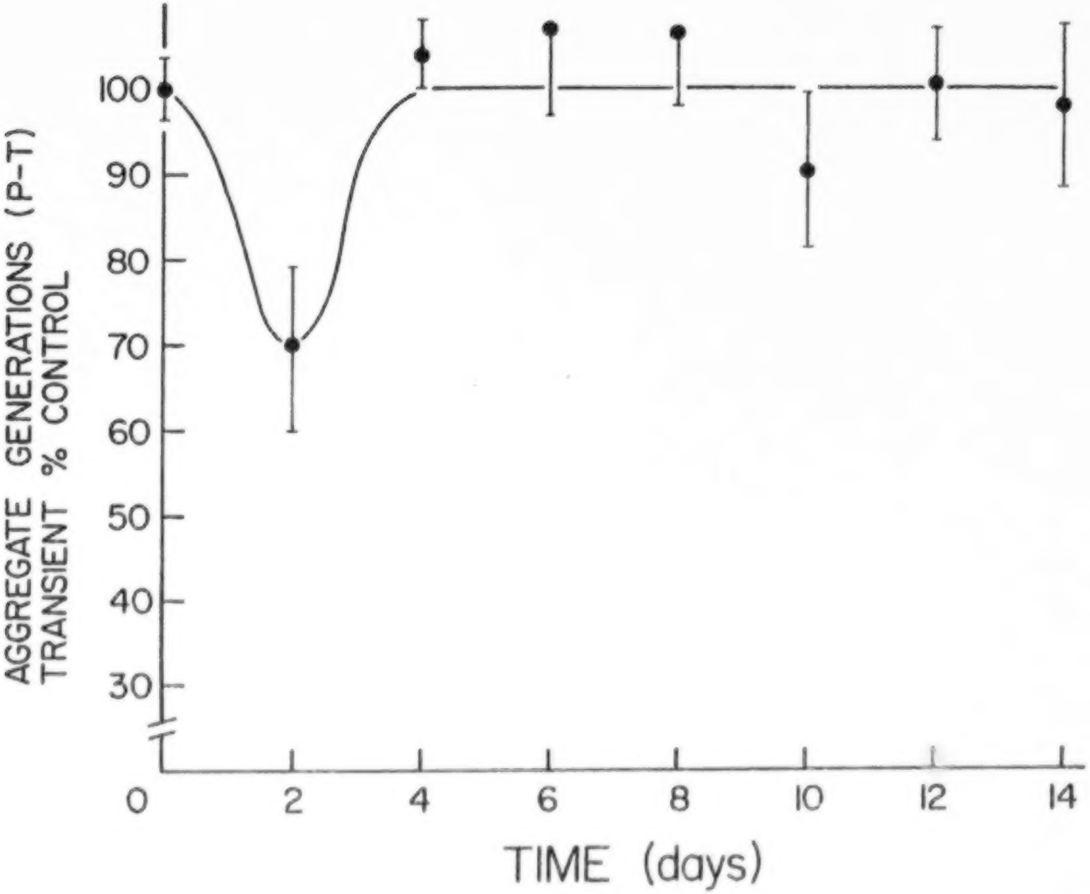

FIGURE 10 DEPURATION EXPERIMENT, AGGREGATE GENERATIONS (P-T) TRANSIENT PHOTOSYNTHETIC EFFECTS

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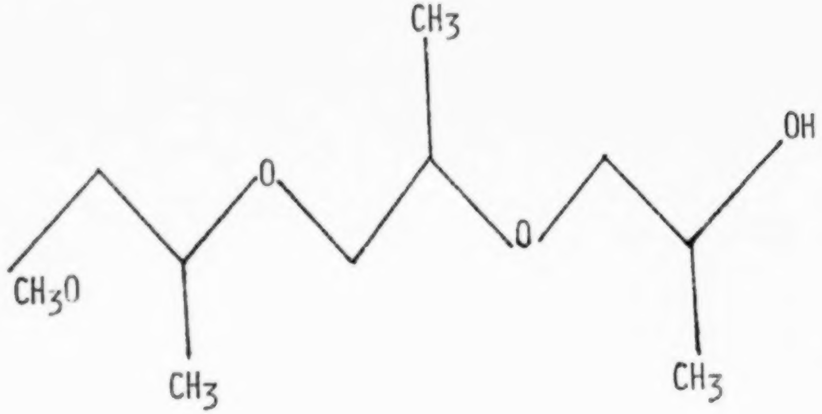
# APPENDIX 1: DOWANOL STRUCTURE

Dowanol TPM Tripropylene Glycol Methyl Ether

Atomic components: $C_{10}H_{22}O_4$

Molecular formula: $CH_3O(CH_2(CH_3)O)_3H$



Molecular weight: 206.3

Boiling point: 242.4°C

# PARTITIONING OF SPRUCE BUDWORM INSECTICIDES IN FOREST STREAMS

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EIDT, D.C. 1985. Partitioning of spruce budworm insecticides in forest streams. Can. Tech. Rep. Fish. Aquat. Sci. 1368: p. 289.

Fenitrothion and aminocarb from either aqueous or oil-based formulations mix equally rapidly with turbulent streamwater. Fenitrothion was taken up in sediments and on suspended material in the water, but aminocarb was not. In sediment, fenitrothion partitioned to the organic fraction. Concentrations of fenitrothion were briefly above prespray levels in most plants and insects sampled, and in some above peak concentrations found in the water. Aminocarb on the other hand, was found in plant and insect tissue only occasionally and in trace amounts. The evidence from these experiments indicates no difference in the rates of diminuation of concentration downstream.

EIDT, D.C. 1985. Partitioning of spruce budworm insecticides in forest streams. Can. Tech. Rep. Fish. Aquat. Sci. 1368: p. 289.

Le fenitrothion et l'aminocarbe contenus dans des formules aqueuses ou à base d'huile se mélangent avec la même rapidité à l'eau des rivières agitées. Le fenitrothion a été fixé sur les sédiments et sur les particules en suspension dans l'eau, mais pas l'aminocarbe. Dans les sédiments, le fenitrothion s'est dispersé dans la fraction organique. Les concentrations de fenitrothion ont dépassé brièvement les niveaux d'avant pulvérisation dans la plupart des plantes et des insectes recueillis et, dans certains cas, les pics de concentration observés dans l'eau. Par contre, on n'a trouvé l'aminocarbe dans les tissus des plantes et des insectes qu'occasionellement et à l'état de trace. Les résultats de nos expériences n'indiquent aucune différence du taux de diminution de la concentration vers l'aval.

# SECONDARY EFFECTS ASSOCIATED WITH TREATMENT OF ARTIFICIAL FRESHWATER SYSTEMS WITH TWO MOSQUITO LARVICIDES - CHLORPYRIFOS AND CHLORPYRIFOS-METHYL

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HEBDA, A.J. and M.G. BOYER. 1985. Secondary effects associated with treatment of artificial freshwater systems with two mosquito larvicides - chlorpyrifos and chlorpyrifos-methyl. Can. Tech. Rep. Fish. Aquat. Sci. 1368: p. 291.

Application of Mosquito larvicides to freshwater systems has been shown to cause secondary effects in the form of significant algal blooms at varied periods of time post-treatment. Chemical, physical and biological parameters were monitored during and after the treatment of artificial freshwater ponds with Chlorpyrifos and Chlorpyrifos-methyl, two organophosphorus pesticides. Laboratory experiments were carried out to monitor release of nutrients from dead and dying invertebrates. A possible mechanism for the occurrence of the post-treatment algal blooms as they related to nutrient availability is postulated.

HEBDA, A.J. and M.G. BOYER. 1985. Secondary effects associated with treatment of artificial freshwater systems with two mosquito larvicides - chlorpyrifos and chlorpyrifos-methyl. Can. Tech. Rep. Fish. Aquat. Sci. 1368: p. 291.

L'application de larvicides contre les moustiques sur des systèmes d'eau douce a présenté des effets secondaires sous forme d'importantes poussées prolifératives d'algues à différentes périodes de temps après traitement. Les paramètres physiques, chimiques et biologiques ont été surveillés pendant et après traitement de mares artificielles d'eau douce avec du Chlorpyrifos et du Chlorpyrifos-méthyl, deux insecticides organophosphorés. Des expériences de laboratoire ont servi à surveiller la libération de substances nutritives à partir d'invertébrés morts ou en train de mourir. On pourrait envisager un mécanisme intervenant dans l'apparition d'une poussée proliférative d'algues après traitement qui soit en rapport avec la disponibilité des substances nutritives.

# THE EFFECTS OF PENTACHLOROPHENOL ON THE PHYSIOLOGY AND BEHAVIOR OF YOUNG-of-YEAR LARGEMOUTH BASS, MICROPTERUS SALMOIDES

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JOHANSEN, P.H., R.A. MATHERS, J.A. BROWN, P.W. COLGAN, and W.G. KIERSTEAD. 1985. The effects of pentachlorophenol on the physiology and behavior of young-of-year largemouth bass, Micropterus salmoides. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 293-295.

The broad objective of our research is to determine the effects of chronic exposure to low concentrations of water borne toxic chemicals on the behavior and physiology of young-of-year fish.

Pentachlorophenol (PCP) is a general metabolic poison that acts by uncoupling oxidative phosphorylation. It is one of several chlorophenols used extensively as biocides, especially in wood preservation, classed as priority chemicals requiring further study of their environmental impact. PCP, the most toxic of the class, is found at low concentrations in several water bodies including the Great Lakes. This study examines effects of PCP on early life stages of largemouth bass (Micropterus salmoides).

Young fish tend to be most susceptible to pollutants. It is during the early stage of free swimming that adequate nutrition is essential and the behavior of food acquisition progressively develops. Since this critical stage is so important to survival, and especially survival over the first winter, we have focused this season's study on the effects of PCP on the development of feeding behavior and growth.

The bass were collected from the nests shortly after hatching and placed in PCP solutions from a continuous flow proportional diluter at concentrations of 0.03, 0.9, 7.5, 41.0 and 83.0 µg/L and untreated water. These concentrations range from concentrations found in Lake Ontario water (0.01 to 0.04 µg/L) to 50% of the 96 h LC50.

Additional fry were reared at 25°C and periodic determinations of the 96 h LC50 made. These tests were undertaken in aerated static PCP solutions that were changed once daily after feeding. Dead fish were removed during frequent daily checks. The LC50 data were analyzed using GLIM computer statistical program.

Based upon data collected to date we conclude that:

(1) Largemouth bass fry under 11.0 mm total length (less than 30 days post swim-up) are more tolerant ($p<0.05$) of PCP poisoning (96 h LC50 of 275 to 287 µg/L) than larger (up to 55 mm) and older (up to 84 days post swim-up) bass (96 h LC50 of 136 to 189 µg/L).

(2) Feeding behavior over the first 5 weeks undergoes developmental changes. Initially this behavior consists of 5 discrete acts, one of which, orientation, is reduced in frequency of occurrence by chronic exposure to PCP at 41.0 and 83.0 µg/L. The differences are most evident after 3 weeks of exposure. Fish exposed to the higher concentrations of PCP are generally more lethargic than untreated fish and those exposed to concentrations below 7.5 µg PCP/L.
(3) Growth is significantly ($p < 0.05$) retarded by exposure to PCP at 7.5 µg/L and higher.
(4) Over a 17 week period there was a statistically significant ($P < 0.05$) dose-related mortality for fish chronically exposed to low concentrations of PCP.
(5) Chronic PCP exposure appears to enhance the susceptibility of young bass to infection by the protozoan *Ichthypophthirius multifiliis*, but not in a dose dependent fashion.

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JOHANSEN, P.H., R.A. MATHERS, J.A. BROWN, P.W. COLGAN, and W.G. KIERSTEAD. 1985. The effects of pentachlorophenol on the physiology and behavior of young-of-year largemouth bass, *Micropterus salmoides*. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 293-295.

L'objectif général de nos recherches était de déterminer les effets de l'exposition à long terme à de faibles concentrations de produits chimiques toxiques transportés par l'eau sur le comportement et la physiologie des jeunes poissons de l'année.

Le pentachlorophénol (PCP) est un poison métabolique général qui agit en découplant la phosphorylation oxydative. Il représente un des nombreux chlorophénols utilisés de façon extensive comme biocide, spécialement pour la préservation des bois, et il est classé parmi les produits chimiques prioritaires dont les effets sur l'environnement doivent être étudiés à fond. Le PCP, qui est le plus toxique de sa catégorie, se trouve à de faibles concentrations dans plusieurs plans d'eau, incluant les Grands lacs. L'objet de notre étude était d'examiner les effets du PCP sur les premiers stades de la vie de l'achigan à grande bouche (*Micropterus salmoides*).

Le jeune poisson a tendance à être plus sensible aux polluants. C'est au premier stade de la période de nage libre qu'une nutrition appropriée est essentielle et que se développe progressivement le comportement d'acquisition de nourriture. Du fait que ce stade critique est si important à la survie, et surtout à la survie après le premier hiver, nous avons concentré le travail de la saison sur les effets du PCP sur le développement du comportement alimentaire et sur la croissance.

Les achigans ont été retirés de leur nid peu après l'éclosion des oeufs, et placés dans des solutions de PCP provenant d'un diluant proportionnel à flux continu, aux concentrations de 0,03, 09, 7,5 41,0 et 83,0 µg/L et de l'eau non traitée. Ces concentrations allaient donc des concentrations trouvées dans le lac Ontario (0,01 à 0,04 µg/L) jusqu'à la moitié de la CL50 - 96h.

De plus, de jeunes poissons ont été élevés à 25°C et on a procédé à des déterminations périodiques de la CL50 - 96h. Ces tests se sont poursuivis dans des solutions statiques aérées de PCP, changées une fois par jour après nourrissage. Les poissons morts étaient retirés à l'occasion de fréquentes vérifications journalières. Les données de CL50 ont été analysées au moyen d'un programme statistique informatique GLIM.

En fonction de ces données, nous parvenons aux conclusions suivantes:

(1) Le jeune achigan à grande bouche, d'une longueur totale inférieure à 11,0 mm (moins de 30 jours après la montée en surface) tolère mieux ($p<0,05$) l'empoisonnement au PCP (CL50 - 96h de 275 à 287 µg/L) que les grands (jusqu'à 55 mm) et les plus vieux (jusqu'à 84 jours après la montée en surface) (CL50 - 96h de 136 à 189 µg/L).

(2) Le comportement alimentaire au cours des 5 premières semaines subit des transformations. Initialement, il comporte 5 actes distincts dont l'un, l'orientation, est réduit, en fréquence, par l'exposition de longue durée au PCP à des concentrations de 410 et 80,0 µg/L. Les différences sont les plus apparentes après 3 semaines d'exposition. Le poisson exposé à de plus fortes concentrations de PCP est généralement plus léthargique que le poisson non traité et que celui qui est exposé aux concentrations inférieures à 7,5 µg de PCP/L.

(3) La croissance est retardée de façon significative ($p<0,05$) par l'exposition au PCP à des concentrations égales ou supérieures à 7,5 µg/L.

(4) Sur une période de 17 semaines, on a observé une mortalité statistiquement significative ($p<0,05$), en fonction de la dose, chez les poissons exposés de façon chronique à de faibles concentrations de PCP.

(5) L'exposition chronique au PCP semble accroître la vulnérabilité des jeunes achigans à l'infection par le protozoaire Ichthypophthirius multifilliis, mais d'une façon qui n'est pas directement reliée à la dose.

# PHYTOTOXICITY TESTING WITH COMMON DUCKWEED (LEMNA MINOR)

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LOCKHART, W.L., B.N. BILLECK, and G.W. BUCHKO. 1985. Phytotoxicity testing with common duckweed (Lemna minor). Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 297-298.

Aquatic macrophytes contribute the major proportion of primary productivity in many shallow freshwater locations; and they provide essential habitat for aquatic and terrestrial animal species. Relatively little literature has been published describing either the susceptibility of macrophytes to chemical exposures, or the tendency of these plants to accumulate chemicals. Common duckweed has often been studied in botanical laboratories, and clones are easily maintained in axenic culture. Early work on the development of phenoxy herbicides used duckweed successfully as a bioassay organism, and it is still used in development of new chemicals, but the results seldom appear in the open literature.

We have used duckweek for phytotoxicity studies with herbicides and also to describe the bioconcentration of organic compounds from exposure media. This report will describe some of our results using culture growth curves as a bioassay response, and our use of Zitko's and other equations to describe bioconcentration. In addition, it will describe our first attempts to use duckweed plants in studies of multiple simultaneous exposures to different chemicals. We have exposed the plants to varous mixtures of Normal Wells crude oil and different dispersants being considered for potential freshwater use, and described growth responses.

Generally the growth responses of the cultures can be well described by equations of the form $\log N = a + bt\hat{2} + c$, where $N$ = frond number at time $t$, and where $a$, $b$, and $c$ are empirical constants generated by regression calculations. Herbicide chemicals tend to reduce culture growth in a dose-dependent manner, and this is reflected in dose-related changes in coefficients a and b. Zitko's equations or alternate statistical expressions allow description of bioconcentration of non-polar organic compounds. Growth and bioconcentration descriptions can then be used to predict the growth effect of a given accumulation of an individual compound within plant tissue.

Factorial experiments with oil and dispersant mixtures show different growth responses with different dispersants, but generally the growth effect seems to be due to oil rather than dispersant, with some dispersants able to ameliorate the oil response more effectively than others.

LOCKHART, W.L., B.N. BILLECK, and G.W. BUCHKO. 1985. Phytotoxicity testing with common duckweed (Lemna minor). Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 297-298.

C'est aux macrophytes aquatiques qu'est attribuable la plus grande proportion de la productivité primaire dans de nombreux plans d'eau douce peu profonds; de plus, ce sont eux qui fournissent l'habitat essentiel aux espèces aquatiques et terrestres. Dans la documentation scientifique, on trouve relativement peu de publications décrivant soit la vulnérabilité des macrophytes aux expositions chimiques, soit la tendance de ces plantes à accumuler les produits chimiques. La lentille d'eau douce a souvent été étudiée dans les laboratoires de botanique et il est facile d'en développer des clones dans des cultures axéniques. Les premiers travaux sur le développement des herbicides phénoxies avaient utilisé avec succès la lentille d'eau comme organisme de dosage biologique et cette plante est toujours utilisée pour le développement de nouveaux produits chimiques; mais les résultats de ces études sont rarement publiés.

Nous avons utilisé la lentille d'eau dans des études de phytotoxicité portant sur des herbicides, ainsi que pour décrire la bioconcentration des produits organiques provenant des milieux d'exposition. Dans notre présentation, nous décrivons certains de nos résultats au moyen de courbes de croissance de cultures comme réaction au dosage biologique, et en utilisant les équations de Zitko et autres pour décrire la bioconcentration. De plus, nous décrirons nos premières tentatives d'utilisation de la lentille d'eau pour étudier les expositions multiples simultanées à des produits chimiques différents. Nous avons exposé les plantes à divers mélanges de pétrole brut du puits Norman, ainsi qu'à divers produits de dispersion dont l'utilisation est envisagée en eau douce, et nous avons décrit les réactions de croissance.

D'une façon générale, les réactions de croissance des cultures peuvent être décrites correctement par des équations du genre $\log N = a + bt^2 + c$, où $N$ = le nombre de frondes au moment $t$, et où $a$, $b$ et $c$ sont des constantes empiriques tirées de calculs de régression. Les produits chimiques herbicides tendent à réduire la croissance des cultures, d'une façon directement reliée à la dose, qui se traduit par des variations reliées à la dose des coefficients $a$ et $b$. Les équations de Zitko, ainsi qui d'autres expressions statistiques permettent de décrire la bioconcentration de produits organiques non polaires. On peut utiliser alors les descriptions de la croissance et de la bioconcentration afin de prédire l'effet sur la croissance d'une accumulation donnée d'un produit déterminé, dans les tissus végétaux.

Les expériences factorielles avec des mélanges de pétrole et de produits de dispersion montrent des réactions de croissance différentes avec différents produits mais, généralement, l'effet sur la croissance semble être dû au pétrole plutôt qu'aux produits de dispersion, car certains de ceux-ci peuvent améliorer la réaction au pétrole plus efficacement que d'autres.

# ZINC DYNAMICS IN THE WINTER FLOUNDER: INFLUENCE OF SEX, SIZE, SEASON AND SAMPLING SITE ON TISSUE CONCENTRATIONS

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SHEARS, M.A., M. KING, and G.L. FLETCHER. 1985. Zinc dynamics in the winter flounder: influence of sex, size, season and sampling site on tissue concentrations. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 299-300.

A field study was conducted to determine whether tissue zinc concentrations in winter flounder (Pseudopleuronectes americanus) differed between heavy metal contaminated and uncontaminated areas.

Zinc concentrations in all tissues examined were found to be influenced by sex and/or body size and/or season when sampled. This made meaningful comparisons between sampling sites very difficult. Flounder caught in the contaminated areas tended to have higher zinc levels in certain tissues than those in uncontaminated areas. However, the degree of tissue elevation was small suggesting that the flounder may be regulating its' body zinc level.

Experimental data indicate that the flounder does not limit the amount of zinc that is absorbed from the digestive tract. In the event of exposure to elevated levels of zinc in the diet, it is suggested that elimination mechanisms may play a greater role in zinc homeostasis than limitation of gastrointestinal uptake.

The winter flounder exhibits a seasonal biology and this is reflected in its zinc dynamics. Based on the distribution of $^{65}$Z in the flounder following an i.m. injection, there appears to be a seasonal change in the turnover of zinc in the tissues. The loss of $^{65}$Zn from the whole flounder (monitored using a whole-body counter) also appears to change seasonally. When flounder were monitored in the summer feeding period, the rate of $^{65}$Zn loss increased over that seen in the winter non-feeding period (average Biological Half-time--223 and 1510 days, respectively).

SHEARS, M.A., M. KING, and G.L. FLETCHER. 1985. Zinc dynamics in the winter flounder: influence of sex, size, season and sampling site on tissue concentrations. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 299-300.

On a fait une étude sur le terrain afin de déterminer si les concentrations de zinc dans les tissus de la plie rouge (Pseudopleuronectes americanus) étaient différentes dans les zones contaminées par les métaux lourds et dans les zones non contaminées.

Dans tous les tissus examinés, les concentrations de zinc étaient influencées par le sexe, la taille ou la saison de prélèvement. C'est pourquoi il fut très difficile de faire des

comparaisons significatives entre les lieux de prélèvement. Les plies rouges capturées dans les zones contaminées présentaient généralement des niveaux de zinc plus élevés dans certains tissus que les poissons pris dans des zones non contaminées. Cependant l'accroissement de la concentration tissulaire était faible, semblant indiquer que la plie rouge pourrait régulariser elle-même le niveau de zinc dans son organisme.

Les données expérimentales indiquent que la plie rouge ne limite pas la quantité de zinc absorbée à partir de l'appareil digestif. En cas d'exposition à des niveaux élevés de zinc dans le régime, on peut supposer que les mécanismes d'élimination jouent un plus grand rôle dans l'homéostase du zinc que la limitation de l'absorption gastrointestinale.

La plie rouge présente une biologie saisonnière, qui se reflète dans la dynamique du zinc. En fonction de la répartition du $^{65}$zinc chez la plie rouge à la suite d'une injection intramusculaire, il semble qu'il y ait une variation saisonnière du métabolisme du zinc au niveau des tissus. La baisse de niveau de $^{65}$Zn chez la plie rouge (contrôlée au moyen d'un compteur pour le corps entier) semble également varier selon les saisons. Lorsque les plies rouges ont été examinées en période de nourrissage estival, le taux d'élimination de $^{65}$Zn a dépassé celui qu'on observe en période hivernale de non-alimentation (demi-vies biologiques moyennes de 225 et 1 510 jours, respectivement).

# PLASMA CORTISOL AND GLUCOSE, AND LIVER GLYCOGEN LEVELS IN STARVED AND FED JUVENILE COHO SALMON (ONCHORHYNCHUS KISUTCH) IN CONSTANT AND DAILY FLUCTUATING TEMPERATURES

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THOMAS, R.E., J.A. GHARRETT, M.G. CARLS, and S.D. RICE. 1985. Plasma cortisol and glucose and liver glycogen levels in starved and fed juvenile Coho salmon (Onchorhynchus Kisutch) in constant and daily fluctuating temperatures. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 301-303.

Clear-cut logging removes streamside vegetation and results increased amplitude in daily temperature fluctuations in streams of southeastern Alaska. In southeastern Alaska, the maximum daily temperature and the daily temperature fluctuation are not as extreme as those encountered in clear-cut forests to the south. Mean summer temperatures in two similar streams on Prince Of Wales Island (southeastern Alaska) differed by only 1.2 C; however, the daily mean fluctuation in the clear-cut stream was over 6 C, double the mean daily fluctuation in the old-growth stream. The maximum daily temperature fluctuation was 9.1 C in the clear-cut stream and 4 C in the old growth stream. The objective of this study was to determine if increased daily temperature fluctuations were stressful to juvenile coho salmon.

Juvenile coho salmon (Oncorhynchus kisutch) were exposed for up to 19 days to four temperature regimes, all of which had a mean of 11 C; daily temperatures cycles (6.5-20 C, 9-15 C, 10-13 C) and a stable temperature of 11 C. In short-term tests, plasma cortisol and glucose were measured every 3 h for 2.4 days to determine the timing of acute stress responses and the presence or absence of diurnal rhythms. In long-term 19 day tests, two groups of fish were tested in each temperature regime: starved and fed. Temperature induced stress was determined by periodically measuring plasma cortisol and glucose concentrations. Condition of the fish was estimated by measuring body weight, liver weight, and liver glycogen. The maximum temperature cycle that acclimated coho could tolerate was 4-26.5 C.

In the short-term tests, acute stress responses were not observed. Plasma cortisol concentrations were not affected, and did not even have a diurnal rhythm, in spite of the strong environmental cues of fluctuating temperatures and light-dark cycles. Plasma glucose concentrations did elevate in the fish from the highest temperature cycles when the temperatures peaked. The glucose concentrations did not elevate to stress levels, like those observed in handling stress, but probably reflected changes associated with changes in metabolism at the high temperatures. In long-term tests, plasma cortisol and glucose concentrations were significantly greater in fish exposed to the most extreme daily temperature fluctuations. Smaller fluctuations in temperature and constant temperature did not induce cortisol differences, even in starved fish. Although cortisol concentrations were elevated, they were not at concentrations generally associated with acute stress.

Body weight, liver weight, and liver glycogen levels were not affected by temperature regimes, but were rapidly affected by starvation. Only 3 days of starvation were required before liver weights decreased. Starvation did not enhance the effects of fluctuating temperatures.

Extreme daily temperature fluctuations, which might be encountered in streams associated with clear-cut logging, may be stressful, as measured by plasma cortisol concentrations. However, over a short 19-day period, this degree of temperature stress alone is not debilitating.

THOMAS, R.E., J.A. GHARRETT, M.G. CARLS, and S.D. RICE. 1985. Plasma cortisol and glucose and liver glycogen levels in starved and fed juvenile Coho salmon (Onchorhynchus Kisutch) in constant and daily fluctuating temperatures. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 301-303.

Les coupes à blanc suppriment la végétation en bordure des cours d'eau, ce qui entraîne un accroissement d'amplitude dans les fluctuations journalières de la température, dans les cours d'eau du sud-est de l'Alaska. Dans cette région la température journalière maximale et les variations journalières ne sont pas aussi extrêmes que celles qu'on rencontre dans les forêts coupées à blanc du sud. Les températures estivales moyennes de deux cours d'eau similaires de l'île Prince-de-Galles (sud-ouest de l'Alaska) différaient de 1,2°C seulement; pourtant, la fluctuation journalière moyenne du cours d'eau dont la végétation riveraine a été coupée à blanc a dépassé 6°C, soit le double de la fluctuation journalière moyenne de cours d'eau aux rives boisées. La fluctuation de température journalière maximale a été de 9,1°C dans le cours d'eau aux rives coupées à blanc et de 4°C dans le cours d'eau aux rives boisées. L'objectif de cette étude a été de déterminer si l'augmentation des fluctuations de température journalière constituait un facteur de stress pour le jeune saumon coho.

De jeunes saumons cohos (Onchorhynchus kisutch) ont été exposés, pendant des périodes allant jusqu'à 19 jours, à quatre régimes de températures ayant chacun une moyenne de 11°C des cycles journaliers (6,5-20°C, 9-15°C, 10-13°C) et une température stable de 11°C. Pendant les tests à court terme, le glucose et le cortisol plasmatiques ont été mesurés à toutes les heures pendant 2,4 jours, afin de déterminer le moment des réactions au stress aigu et la présence ou l'absence de rythmes diurnes. Pour les tests à long terme, s'étendant sur 19 jours, deux groupes de poissons ont été étudiés à chaque régime de températures: des poissons non nourris et des poissons nourris. Le stress induit par la température a été déterminé par la mesure périodique des concentrations plasmatiques de cortisol et de glucose. L'état des poissons a été estimé par mesure du poids corporel du poids du foie et du niveau de glycogène hépatique. Le cycle de températures maximal que le coho acclimaté pouvait tolérer était de 4-26,5°C.

Dans les tests à court terme, on n'a pas observé de réaction au stress aigu. Les concentrations de cortisol plasmatique n'ont pas été modifiées, et n'ont même pas présenté de rythme diurne, en dépit des vigoureuses incitations environnementales constituées par les fluctuations de température et les cycles de lumière et d'obscurité. En fait, les concentrations de glucose plasmatique se sont élevées chez les poissons soumis aux cycles de températures extrêmes, au moment des sommets de température. Les concentrations de glucose ne se sont pas élevées au niveau de stress comme celles qu'on

observe dans le stress de manipulation, mais ont probablement traduit des modifications associées aux variations de métabolisme à des températures élevées. Dans les tests à long terme, les concentrations de cortisol et de glucose plasmatiques ont été significativement plus grandes chez les poissons exposés aux plus extrêmes fluctuations de température journalières. Les fluctuations de température limitées et les températures constantes n'ont pas amené de différence du niveau de cortisol, même chez les poissons non alimentés. Bien qu'atteignant des niveaux élevés, ces concentrations ne pouvaient être associées, d'une façon générale, avec un stress aigu.

Le poids corporel, le poids du foie et les niveaux de glycogène hépatique n'ont pas été modifiés par des régimes de température, mais ils ont été rapidement influencés par le manque de nourriture. Trois jours de jeûne seulement suffisaient pour qu'on observe une diminution du poids du foie. L'absence de nourriture n'a pas augmenté les effets des fluctuations de température.

Les fluctuations de température journalières extrêmes telles qu'on peut les rencontrer dans les cours d'eau dont la végétation riveraine a subi des coupes rases, peuvent causer des stress, ainsi qu'on peut le mesurer par des concentrations de cortisol plasmatique. Cependant, sur une courte période de 19 jours, ce type de stress de température, à lui seul, n'est pas fragilisant.

# CONTRIBUTED PAPERS - VARIOUS TOPICS

R. Wilson, Chairman
B. Ernst, Chairman

# AN EC50 ALGAL GROWTH INHIBITION MICROTEST USING ATP MEASUREMENTS

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BLAISE, C., R. LEGAULT, and N. BERMINGHAM. 1985. An EC50 algal growth inhibition microtest using ATP measurements. Can. Tech. Rep. Fish. Aquat. Sci. 1368: p. 305.

An attempt to develop an algal microtest to determine EC50's using ATP as the parameter of choice was undertaken. This study is consequential to an earlier investigation where a conventional and miniaturized assay, respectively making use of flasks and microplates as culture vessels, had been compared by cell count measurements. Because microplates are attractive in conducting algal bioassays and since ATP, as a parameter, presents definite advantages over cell counts obtained with electronic particle counters, interest to couple these two features triggered this on-going work. Experimental procedure of the microtest is described and comparative data obtained with conventional and microplate assays measured by cell counts are presented with industrial effluent samples as testing material.

BLAISE, C., R. LEGAULT, and N. BERMINGHAM. 1985. An EC50 algal growth inhibition microtest using ATP measurements. Can. Tech. Rep. Fish. Aquat. Sci. 1368: p. 305.

Nous avons entrepris de mettre au point un microtest sur les algues visant à déterminer la CE50 en prenant l'ATP comme paramètre de choix. Cette étude fait suite à une recherche antérieure au cours de laquelle nous avons comparé par numération cellulaire un essai conventionnel et un essai miniaturisé, utilisant respectivement des flacons et des microplaques comme contenants de culture. Les microplaques constituant un moyen commode de faire des dosages biologiques sur algues, et l'ATP, pris comme paramètre, présentant des avantages certains sur les numérations cellulaires obtenues par des compteurs électroniques de particules, nous avons trouvé intéressant de rassembler ces deux éléments pour entreprendre la présente étude. Nous décrivons la procédure expérimentale du microtest, en donnant les données comparatives obtenues avec les dosages conventionnels et les dosages sur microplaques comportant des numérations cellulaires, appliqués à des échantillons d'effluent industriel.

# A RAPID TECHNIQUE FOR DETERMINING TOXICANT EFFECTS ON A GREEN ALGA

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BURRELL, R.E., C.I. MAYFIELD, W.E. INNISS and K. KUMMER 1983 A rapid technique for determining toxicant effects on a green alga. Can. Tech. Rep. Fish. Aquat. Sci. pp. 307-313.

Growth rates and total yield were measured spectrophotometrically for *Ankistrodesmus bralunii*, grown under previously determined optimal conditions, and compared to the growth rate and total yield of cells grown under standard algal assay conditions. Cells grown under the standard algal assay conditions had much slower growth rates than those grown under the optimized conditions and after 12 days, total yield was 1.2 % and 4.6 % of that in the optimal heterotrophic and autotrophic treatments respectively. Cells grown under the optimal autotrophic conditions had a yield which was 27 % of that in the optimal heterotrophic conditions. Since the evidence suggested that both the optimized autotrophic and heterotrophic systems might yield toxicity data more rapidly than the standard algal assay, the response of *A. braunii* to the photosynthetic inhibitor atrazine was determined. These responses were compared to that obtained with the standard algal assay. Slow growing cells of the standard algal assay procedure resulted in an $EC_{50}$ of 0.095 ug mL$^{-1}$ of atrazine while rapidly growing cells of the optimized autotrophic and heterotrophic procedure gave $EC_{50}$ values of 0.05 ug mL$^{-1}$ and 2.2 ug mL$^{-1}$ respectively. While all $EC_{50}$ values were significantly different, the optimized autotrophic and the standard algal assay systems were of the same order of magnitude. The close approximation of these two systems indicated that the optimal autotrophic test system resulted in acceptable estimates of toxicity.

BURRELL, R.E., C.I. MAYFIELD, W.E. INNISS and K. KUMMER 1983 A rapid technique for determining toxicant effects on a green alga. Can. Tech. Rep. Fish. Aquat. Sci. pp. 307-313.

Le taux de croissance et la production totale de l'*Ankistrodesmus braunii* cultivé dans des conditions optimales déterminées à l'avance ont été mesurés à l'aide d'un spectrophotomètre et comparés au taux de croissance et à la production des cellules cultivées dans des conditions normales d'analyse des algues. Les cellules cultivées dans les conditions normales d'analyse des algues ont montré des taux de croissance très inférieurs aux taux de croissance des cellules cultivées dans les conditions optimales. Après 12 jours, la production totale était 1.2 % et 4.6 % de la production dans les traitements optimaux hétérotrophe et autotrophe respectivement. La production des cellules cultivées dans des conditions optimales autotrophes représentait 27 % de la production obtenue dans des conditions optimales hétérotrophes. Puisque les résultats suggéraient que les deux systèmes optimaux, soit hétérotrophe et autotrophe, produisaient des résultats plus rapidement que l'analyse normale des algues, les réactions d'*A. braunii* à l'inhibiteur photosynthétique atrazine ont été déterminées. Ces réactions ont été

comparées aux réactions obtenues par analyse normale des algues. Les cellules à croissance lente de l'analyse normale des algues avaient des valeurs $EC_{50}$ de 0.095 ug $mL^{-1}$ d'atrazine tandis que les cellules à croissance rapide résultant des traitements optimaux autotrophe et hétérotrophe avaient des valeurs $EC_{50}$ de 0.06 ug $mL^{-1}$ et 2.2 ug $mL^{-1}$ respectivement. Quoiqu'il y eut une différence significative entre les deux valeurs $EC_{50}$, les résultats du traitement optimal autotrophe et de l'analyse normale étaient du même ordre de magnitude. L'approximation rapprochée des deux systèmes indiquait que le traitement optimal autotrophe estimait la toxicité de façon acceptable.

## INTRODUCTION

The development of a rapid accurate technique for the evaluation of toxicant effects on algae is essential (EPA, 1978). At present, routinely used batch type algal toxicant assays require incubation periods of 16 days (Fisher et al., 1974) to 58 days (Kindig, 1979 in Hammons, 1981). Incubation periods of this length are not efficient for screening the large numbers of chemicals which are potentially toxic (Hushon et al., 1979; Trevors et al., 1981). The simplest way to reduce the incubation time required is to increase the growth rate of the test organism. This can be achieved through inorganic (Hemerick, 1973; Ukeles, 1973) or organic supplements (Nielson and Lewin, 1974). Organic supplements, which increase growth rates, may result in biased toxicity data for compounds that block photosynthesis (Conner, 1981). Cells which grow heterotrophically may circumvent photosynthetic pathways thus decreasing observed toxicity.

In this paper, a comparison of atrazine effects is made with a green alga grown in an optimized autotrophic medium and a heterotrophic medium. These results are compared to those obtained in a standard algal assay.

### Materials and Methods

Ankistrodesmus braunii ATTC 12744 was grown in a 500 mL flask containing 300 mL of Bristol medium (Nichols, 1973) supplemented with 0.1 % yeast extract (w/v) and 0.5 % glucose (w/v) on a rotary shaker at 100 rpm for 6 days at 25 ± 1.5°C. Fluorescent agrolite (Westinghouse tubes on a 16h - 8h light - dark cycle provided illumination at an intensity of 30 u Einsteins $m^{-2}$ $s^{-1}$. On day 6 the number of cells $mL^{-1}$ was determined by direct counts with a 0.2-mm hemocytometer (Fuchs-Rosenthal grid) and an appropriate volume was added to each experimental flask to give a final cell count of $1 \times 10^5$ $mL^{-1}$.

Stock solutions of atrazine, 2-chloro-4-ethylamine-6-isopropylamino-1, 3,5-triazine (Chem Service, West Chester, Pa.) were prepared in pesticide quality methanol (Matheson, Coleman and Bell, Norwood, Ohio). Additions of atrazine were made in 0.1-mL volumes to 300-mL volumes of medium.

### Standard Assay

A series of atrazine concentrations of 0, 0.03, 0.05, 0.07 and 0.1 ug $mL^{-1}$ were prepared in 15 500 mL Erlenmeyer flasks containing 300 mL of Bristol solution and stoppered with foam plugs. A. braunii cells were then added and the flasks were incubated at 24° C and on a 16h - 8h light - dark cycle for 27 days. Each flask was shaken once a day. Optical densities (678 nm) were determined on day 27 and an $EC_{50}$ was calculated.

### Rapid Autotrophic

A series of atrazine concentrations of 0, 0.04, 0.06, 0.07 and 0.1 ug $mL^{-1}$ were prepared in triplicate in 15 500-mL Erlenmeyer flasks containing 300 mL of Bristol solution. The flasks were set up for bubbling at 350 mL $min^{-1}$ with $CO_2$-supplemented (0.12 % v/v) air. A. braunii was inoculated ($1 \times 10^5$ $mL^{-1}$) and the flasks were incubated at 24° C on a 16h -8h light-dark cycle for 11 days. Optical densities were determined on day 11 and an $EC_{50}$ was calculated.

### Rapid Heterotrophic

A series of atrazine concentrations of 0, 0.5, 1.0, 2.0 and 10.0 ug $mL^{-1}$ were prepared in triplicate in 15 500-mL Erlenmeyer flasks containing 300 mL of Bristol solution amended with 0.1 % yeast extract (w/v) and 0.5 % glucose (w/v). The flasks were equipped for bubbling at 350 mL $min^{-1}$ with $CO_2$-supplemented (0.12 % (v/v) air. A. braunii was inoculated ($1 \times 10^5$ $mL^{-1}$) and the flasks were incubated at 24° C on a 16h - 8h light-dark cycle for 11 days. On day 11, optical densities were determined and an $EC_{50}$ was calculated.

### Growth Curves

Spectrophotometric readings of samples taken periodically from the control flasks of each assay were plotted to give growth curves of the alga for each set of growth conditions.

### Statistics

Student t test (two tailed) (Zar, 1974) was used for the comparison of experimental means. Dunnett's multiple t test (Zar, 1974) was used for comparison of experimentals to controls.

## RESULTS AND DISCUSSION

Growth curves for A. braunii grown autotrophically in Bristol medium, Bristol medium + $CO_2$ and heterotrophically in Bristol medium + $CO_2$ + 0.1 % yeast extract + 0.5 % glucose (HGM) are shown in Figure 1. The data clearly indicate that cells grown heterotrophically have much higher growth rates than those grown autotrophically. Cells in the autrophic medium supplemented with $CO_2$ grew faster than cells grown in the static Bristol medium. For bioassays where rapidity is important, the use of a heterotrophic growth medium or an autotrophic medium with supplemental $CO_2$ should be considered.

Toxicity curves for A. braunii and atrazine in static (Bristol), 350 mL $min^{-1}$ air + $CO_2$ (Bristol) and 350 mL $min^{-1}$ air + $CO_2$ (HGM) treatments are presented in Figure 2. The calculated $EC_{50}$ values for the cells of the static and bubbled Bristol treatments were 0.095 and 0.06 ug $mL^{-1}$ respectively. Although these values were significantly different, they were considered reasonable estimates of the actual $EC_{50}$ for the organism considering the radically different growth rates involved. The $EC_{50}$ for the cells of the HGM bubbled treatment was 2.2 ug $mL^{-1}$. This showed that the toxicity of atrazine in HGM was one-twentieth of that observed in the static Bristol treatment. This large difference was probably due to the ability of the cells to bypass photosynthetic pathways in favour of heterotrophic pathways. The same phenomenon was noted with other green algae treated with a polychlorinated biphenyl (Conner, 1981). Due to the ability of the cells to bypass photosynthesis, the general use of HGM in toxicity assays would not be acceptable.

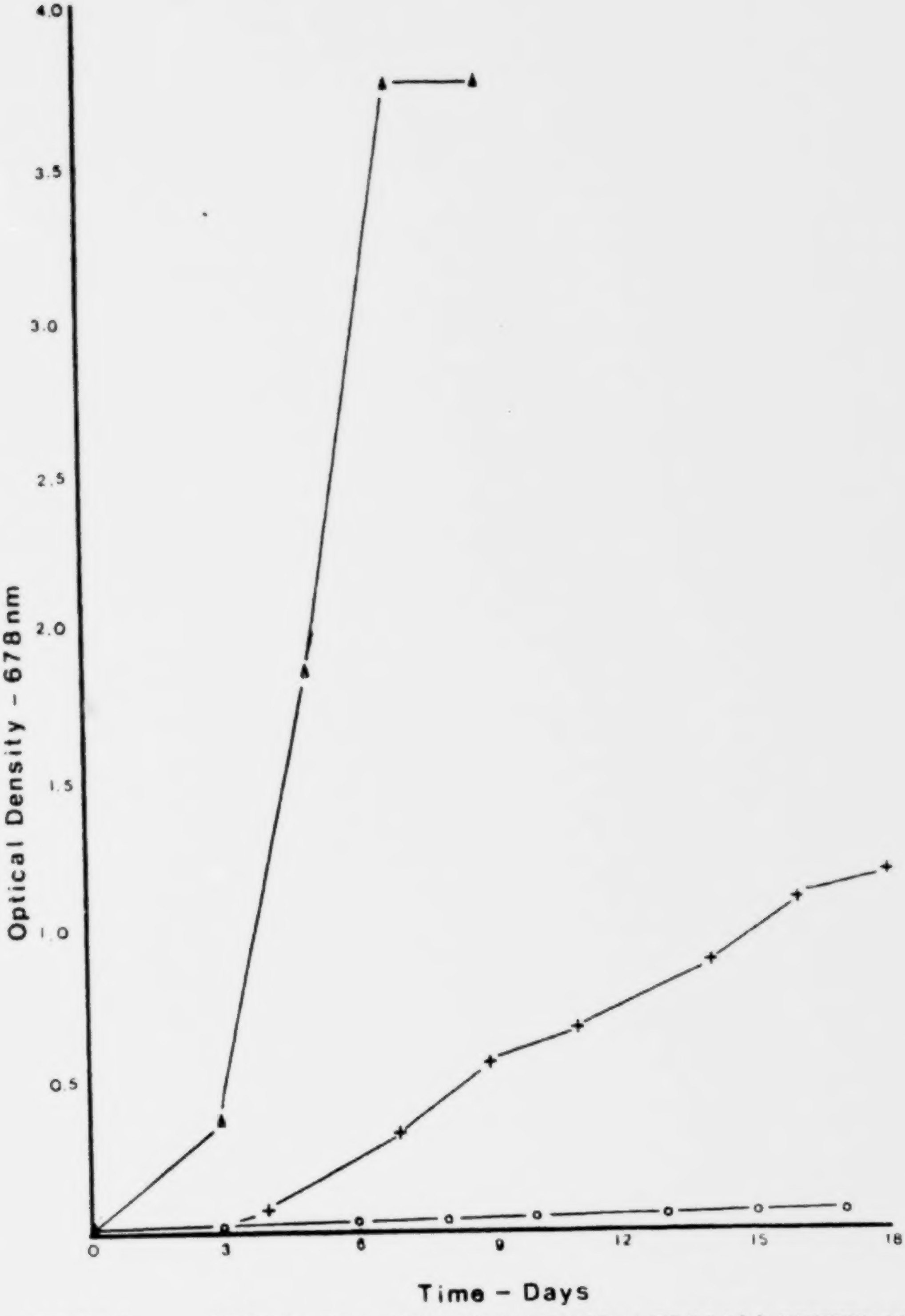

FIGURE 1 GROWTH OF A. BRAUNII IN BRISTOL MEDIUM (o), BRISTOL MEDIUM + $CO_2$ (+) AND BRISTOL MEDIUM + $CO_2$ + 0.1 % YEAST EXTRACT + 0.5 % GLUCOSE (Δ)

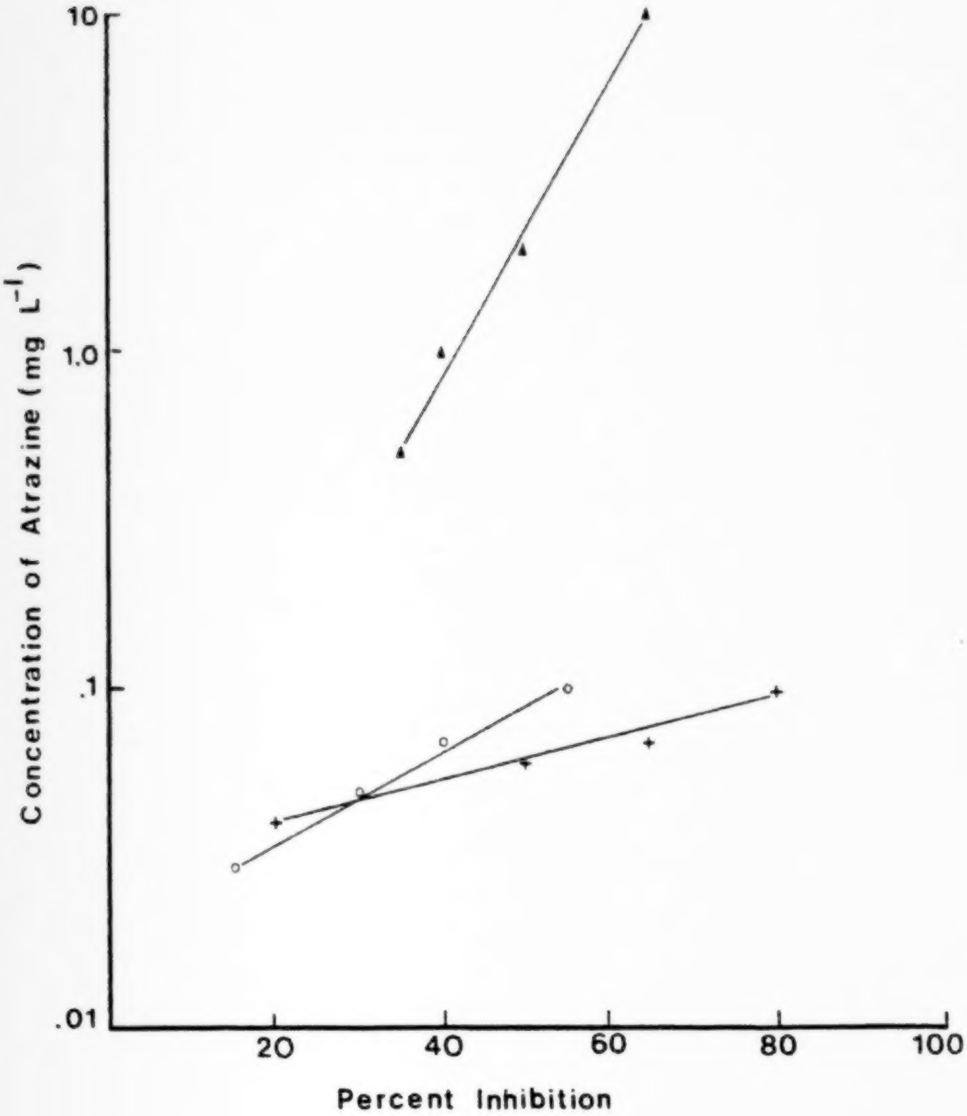

FIGURE 2 TOXIC RESPONSE OF A. BRAUNII TO ATRAZINE IN BRISTOL MEDIUM (DAY 27) (o), BRISTOL MEDIUM + $CO_2$ (DAY 11) (+) AND BRISTOL MEDIUM + $CO_2$ + 0.1 % YEAST EXTRACT + 0.5 % GLUCOSE (Δ)

From these results, it was concluded that HGM should not be used in further toxicity tests. The 350 mL $min^{-1}$ $CO_2$-supplemented treatment was found to be acceptable. the slight difference in toxic response, while undesirable, was more than compensated for by the rapidity of the method. The key point is that these tests are meant to indicate that trends may occur in natural systems. When cells are removed from a natural system to the laboratory, it is difficult, if not impossible, to duplicate the natural system. Thus, toxicity assay results should be considered estimates or trends and not absolute responses.

## AKNOWLEDGEMENT

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# USE OF BIOLOGICAL MONITORING AND ACUTE LETHAL BIOASSAY TESTS AS MEASURES OF AQUATIC TOXICITY - CASE STUDIES AND CRITICAL REVIEW

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CURRIE, R.A. and K.D. PHINNEY. 1985. Use of biological monitoring and acute lethal bioassay tests as measures of aquatic toxicity - case studies and critical review. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 315-319.

The purpose of our presentation is to briefly describe several of the biological monitoring techniques used to detect effluent toxicity for a number of industrial firms in New Brunswick. We will comment on the usefulness and also the weaknesses of these techniques. Four techniques have been used to monitor the toxicity of effluents discharged into receiving streams. These techniques include monitoring of benthic macroinvertebrates, periphyton, fish populations, and acute toxicity bioassays.

CURRIE, R.A. and K.D. PHINNEY. 1985. Use of biological monitoring and acute lethal bioassay tests as measures of aquatic toxicity - case studies and critical review. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 315-319.

L'objet de notre présentation est de décrire brièvement plusieurs des techniques de surveillance biologique que nous utilisons pour déceler la toxicité des effluents d'un certain nombre d'entreprises industrielles du Nouveau-Brunswick. Nous examinerons l'utilité ainsi que les points faibles de ces techniques. Quatre techniques ont servi à contrôler la toxicité des effluents rejetés dans les cours d'eau. Parmi ces techniques : contrôle des macro-inverterbrés benthiques, du périphyton, des populations de poissons, et dosage biologique de toxicité aigue.

## INTRODUCTION

The purpose of our presentation today is to briefly describe several of the biological monitoring techniques we carry-out to detect effluent toxicity for a number of industrial firms in New Brunswick. We will comment on the usefulness and also the weaknesses of these techniques and hopefully elicit comments, in this regard, from those present.

In general, we have used four techniques to monitor the toxicity of effluents discharged into receiving streams. These techniques include monitoring of benthic macroinvertebrates, periphyton, fish populations, and acute toxicity bioassays.

### Benthic Macroinvertebrates

One of the most important biological communities we investigate through our environmental monitoring programs are benthic macroinvertebrates, the animals inhabiting the bottom substracte of streams and lakes. We consider the monitoring of macroinvertebrates to be important because they have been the subject of many studies and their responses to various pollutants are well documented. Ordinarily, a healthy aquatic environment will support a diverse benthic macroinvertebrate population - an abundance of individuals representing a variety of species, with no overabundance of any one group. In the case of a stressed environment, such as one receiving a toxic pollutant, the diversity of species tends to be reduced. Some species may disappear or be noticibly reduced in number, while a few species, not as sensitive to the effects of the pollutant, may proliferate in response to lessened competition or predation.

To measure the effects of a suspected pollutant, we sample benthic macroinvertebrates upstream and downstream from its source. If sampling is conducted in a similar manner in similar habitats, in the control and treatment sites, then one may assume that consistent differences in populations may be due to the effects of the effluent. We collect samples of resident benthic macroinvertebrate populations through the use of natural substrate colonization samplers (similar to a design described by Coleman and Hynes (1970)). Three samplers are imbedded in the stream substrate at each site and permitted approximately 30 days for colonization. After that time the substrate is collected, the organisms are removed and preserved in 10 percent formalin until they are identified and counted.

Benthic macroinvertebrate indices are produced for each site for each sampling interval using the Shannon-Weaver diversity index formula (Poole, 1974). Student's "t" test is then used to statistically compare the indices for the control and treatment sites for each of the sample intervals.

### Periphyton

In response to the request of one of the mining companies, we also collect and have analyzed samples of periphyton, the microscopic plant and animal communities found on stream bottom substrate.

Like macroinvertebrates, certain forms of periphyton are dramatically influenced by pollutants and serve as useful indicators in assessing water quality. Our sampling procedure consists of collecting representative samples upstream and downstream of the minewater outfall by brushing the surfaces of rocks from the stream substrate. The samples are preserved in 5 percent formalin until we have them analyzed. The analysis provides us with such data as species composition and relative abundance as well as community comparisons using both the "Coefficient of Community" and "Percentage of Similarity of Community" tests.

### Fish

In several of our biological monitoring programs we investigate abundance and species composition of resident fish populations. The presence or absence of certain fish species is a useful indication of conditions in the stream environment, for example, salmonids are particularly sensitive to heavy metals and B.O.D. levels.

As with the other life forms, fish populations are sampled upstream and downstream of effluent sources, in similar stream habitats, by standard electrofishing methods. Areas are enclosed with barrier nets and the enclosed area is sampled a series of times, removing the fish each time. Population estimates for each species, and in the case of salmonids for each age class, are produced using both the Zippin and DeLury formulas (Zippin, 1958; DeLury, 1951). The population estimates are compared between sites, and between years, to determine any differences or changes.

### Bioassay Tests

As well as conducting instream monitoring of the environmental effects of effluents to resident organisms, we are also involved in using bioassay tests to determine the toxicity of some of these effluents. The tests are standard 96-hour static bioassay tests and employ standard fish species such as rainbow trout and three-spine sticklebacks.

### General Comments

The monitoring programs we carry-out to detect toxicity of effluents may include any one or more of the techniques described above. This diversity stems from the origin of the monitoring programs: some we design, others are requested by government agencies, while others are specified by the client. Biological monitoring is expensive due to the labour involved in sampling, preparation of samples, and analysis. Thus, for the sake of diversity in the monitoring program, it is often necessary to reduce the intensity and/or frequency of sampling to that less than desireable. This of course leads to less than definitive conclusions to be made for any one set of samples or for any one sampling period. The lack of sufficient may also place in doubt the validity of apparent trends from sample period to sample period.

Thus, where only a limited budget is available it may be more worthwhile to concentrate on one technique of monitoring rather than to attempt to include a diversity of methods for the routine monitoring of the toxicity of effluents.

With respect to our bioassay work, we are currently conducting tests for several New Brunswick based industries. These clients are required to provide government agencies with toxicity results of their effluent on a regular monthly basis, although there is provision to deviate from this schedule as stated in the Metal Mining Liquid Effluent Regulations and Guidelines. We feel that our clients may not be taking full advantage of this provision. In some cases, a reliable indication of toxicity can be obtained at less cost than bioassay tests and on a more frequent basis by comparing past bioassay results to certain characteristics of the effluent.

For example, for two of our clients, the potash mines, we are usually able to closely predict test results before fish are exposed to their effluents. With these tests, mortality of the test fish is closely correlated to basic chemical properties of each material - pH and salinity. Measurements of these parameters could therefore replace some of the bioassay testing and be provided on a more frequent basis as well as more quickly and at significantly less cost to the client.

## CONCLUSIONS

Generally we have found biological monitoring to be a useful tool for detecting and measuring the effects of toxic materials in aquatic environments. To use this tool most effectively requires a knowledge of the best biological communities to study to determine toxic effects, and a monitoring program that will produce conclusive results. However, life is not always that simple. Sometimes we work within such constraints as limited budgets and inflexible study designs which depict the communities we investigate and the frequency of sampling. Such constraints make it extremely difficult to interpret results and form conclusions.

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# USE OF ECOTOXICOLOGICAL AND AVOIDANCE DATA TO ASSESS EFFECTS OF HAZARDOUS MATERIALS ON FISH

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GRAY, R.H., D.D. DAUBLE, and J.R. SKALSKI. 1985. Use of ecotoxicological and avoidance data to assess effects of hazardous materials on fish. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 321-335.

Assessing potential environmental effects from accidental release of hazardous materials to aquatic habitats is often based on laboratory-derived tolerance data and assumptions of organism exposure. This approach usually represents "worst-case" conditions and does not consider the adaptive behavior of mobil organisms such as fish, which may modify actual exposures. We conducted behavioral response studies as part of a larger effort to assess the fate and effects of organically complex, coal-derived liquids in the aquatic environment. A nine-chambered circular apparatus (rosette) was used to test the ability of groups of adult fathead minnow (*Pimephales promelas*) and juvenile rainbow trout (*Salmo gairdneri*) to detect and avoid various concentrations of the water-soluble fraction (WSF) of a coal liquid. Fathead minnow avoided constituent concentrations of the WSF that were acutely toxic, but did not avoid concentrations known experimentally to affect growth and reproduction. In contrast, rainbow trout did not avoid the toxicant at any concentration, despite mortalities of up to 50% of some test groups. A conceptual model is presented that links avoidance and toxicological data, and allows a more realistic environmental assessment than can presently be obtained with toxicity data alone.

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L'évaluation des effets environnementaux possibles du déversement accidentel de produits dangereux pour les habitats aquatiques est souvent basée sur des données de tolérance dérivées des laboratoires et sur des hypothèses d'exposition des organismes. La méthode exposée ici correspond généralement aux conditions de la "pire des hypothèses", et ne tient pas compte du comportement d'adaptation d'organismes mobiles, comme le sont les poissons, qui peut arriver à modifier l'exposition réelle. Nous avons fait des études de réactions comportementales dans le cadre d'un effort plus vaste visant à évaluer le devenir et les effets de liquides organiquement complexes et dérivés du charbon sur l'environnement aquatique. Un appareil circulaire à neuf alvéoles (rosette) a été utilisé pour tester la capacité de groupes de têtes de boule adultes (*Pimephales promelas*) et de jeunes truites arc-en-ciel (*Salmo gairdneri*) de déceler et d'éviter diverses concentrations de la fraction soluble dans l'eau d'un liquide d'origine houillère. Les têtes de boule ont

évité les concentrations de fraction soluble dans l'eau qui étaient extrêmement toxiques, mais n'ont pas évité les concentrations reconnues par expérience comme pouvant perturber la croissance et la reproduction. Par opposition, la truite arc-en-ciel n'a pas évité les produits toxiques, quelles que soient leurs concentrations, en dépit d'une mortalité atteignant 50 % dans certains des groupes. Un modèle conceptuel est présenté, qui relie l'évitement et les données de toxicologie, et permet d'obtenir une évaluation environnementale plus réaliste que celle qu'on peut obtenir actuellement au moyen des seules données de toxicité.

## INTRODUCTION

Assessing the effects of toxic chemicals on aquatic habitats requires the following information: the environmental factors that are, or will be altered, the degree of alteration, the environmental concentration of the released materials, the kinds of organisms present, the sensitivity or tolerance of organisms potentially exposed to the material, and the degree of exposure. During the past several years, numerous protocols have been developed to evaluate acute and chronic toxicity of chemicals to various aquatic and marine organisms. However, information on the behavioral responses of organisms exposed to toxic chemicals and the influence of response on actual exposure is often ignored and thus, represents a missing link in the assessment process (Gray 1983).

Several engineering options to produce liquid fuels from coal have been under development in the United States and may be ready for commercialization by the 1990s. The ecological implications of coal liquid spills during transportation have been under investigation for several years (Gray and Drucker 1981; Strand and Vaughan 1981; Mahlum et al. 1981; Gray and Cowser 1982). Water-soluble fractions (WSFs) derived from coal liquids are toxic to various aquatic species (Bean et al. 1981; Dauble et al. 1982, 1983; Gray et al. 1982; Becker et al. 1983, and others). These WSFs may pose a greater hazard in freshwater habitats than the WSFs of fuel oils presently in commerce (Giddings et al. 1980; Giddings & Washington 1983; Gray et al. 1982; States et al. 1981; Ullrich and Millemann 1983). Toxicological properties of WSFs of coal-derived liquids largely reflect the presence of highly soluble phenolic constituents, whereas the WSFs of petroleum contain primarily aromatic hydrocarbons. Our objective here was to evaluate the behavioral responses of fathead minnow (Pimephales promelas) and rainbow trout (Salmo gairdneri) to various concentrations of coal liquid WSFs and to relate observed behavior to acute and chronic toxicity data.

### Materials and Methods

The behavioral test apparatus consisted of a circular tank with nine peripheral chambers (Gray et al., 1983). The tank was 150 cm in diameter with a center drain that maintained water depths at 25 cm (Figure 1). Water entered from the outside of each peripheral chamber and flowed through a central collection area to the center drain. Test organisms could move throughout the apparatus.

A coal liquid[1] WSF was generated with a mix and separation device (Dauble et al. 1981). The WSF was delivered at desired test concentrations through a dilutor system to the periphery of the test apparatus. Phenols comprised >80% of the total carbon in WSFs; major phenolic constituents were phenol (15%), cresols (37%), C2 phenols (20%), and C3 phenols (9%) (Dauble et al. 1982). Exposure concentrations of phenolics were monitored twice daily by the dye photometric method (APHA 1975). Total phenols determined by gas chromatography were about 40% higher than estimates determined colorimetrically. Filtered Columbia River water was the diluent and flowed to the test system at about 1 l/min.

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[1] A 2.9:1 blend of middle to heavy distillate from the solvent-refined coal (SRC) II pilot plant at Fort Lewis, Washington.

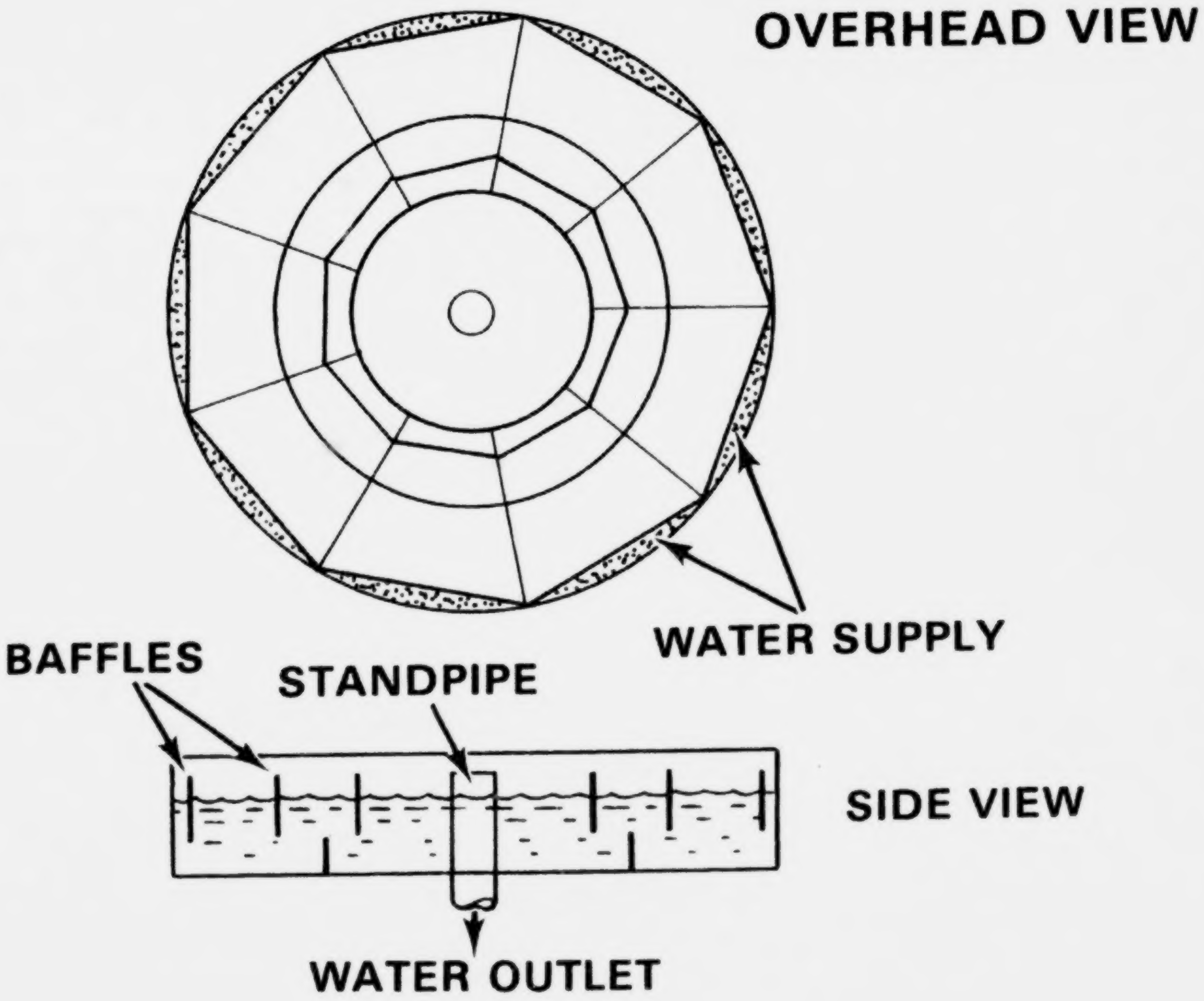
OVERHEAD VIEW
WATER SUPPLY
BAFFLES
STANDPIPE
SIDE VIEW
WATER OUTLET

FIGURE 1 AVOIDANCE CHAMBER (from Gray et al., 1983)

Fish from stocks reared in our laboratory were tested at 12 ± 1° C. Fathead minnow (adults) were 48 to 67 mm fork length (FL); rainbow trout (juveniles) were 80 to 120 mm FL. All observations were made under constant overhead fluorescent lighting. Three replicate test series each involved groups of 36 fathead minnow or 9-36 rainbow trout.

Each test involved choices for test fish in which three chambers each contained a low- and high-toxicant concentration and uncontaminated river water (controls). The center area received equal flows from control, low- and high-toxicant test chambers. The center concentration was similar to that in the low-toxicant chambers. To create a more uniform central mixing zone, no two consecutive chambers had the same concentration.

Initial studies were conducted without toxicant to determine fish response in the test apparatus. Results indicated that fathead minnow generally congregated in one or more groups while rainbow trout were more solitary and territorial. Based on observations of fish dispersal and distribution throughout the chamber over time, we identified three test periods: an acclimation or pre-exposure period of about 24 hours, a transition (after toxicant introduction) and exposure period of 48 hours, and a transition (after toxicant shutdown) and post-exposure period of 24 hours. Fish location in the test system was monitored and recorded on video tape for 30 seconds every 30 minutes with an overhead camera. The test system and camera were shielded from outside room activity in a white cloth enclosure.

Fish distribution data were analyzed by several statistical approaches as described by Dauble et al. (ms submitted) and summarized below. First, a multivariate one-way analysis of variance (ANOVA) was used to test for spatial differences among test periods. The response variable was the number of fish in the control, low-toxicant, high-toxicant and center chambers during each 30-second observation. The mean vector of responses (counts) was compared among the three test periods.

A response variable was also computed from the relative number of fish in control, low-toxicant, high-toxicant and center chambers at each observation. The chamber with the largest number of fish was assigned a value of 1; all other chambers were assigned a zero. Preference indices were summed for all replicate observations during a test period. Converting fish counts to a preference index avoided the problem of non-independent behavior by the fish (i.e., schooling or territoriality). Fish preference was also evaluated with a 3 x 4 chi-square contingency test. The analysis assumed that the 30-second observations were independent and that fish had sufficient time to redistribute themselves if desired. The null hypothesis was that chamber preference (high-toxicant, low-toxicant, control or center) was homogeneous during the three test periods.

Finally, for fathead minnow, a logistic-sigmoid curve (Finney 1978) was fitted to the concentration-response data to allow predictions of avoidance. The model was produced by non-linear least-squares, with log-transformed estimates of the center chamber concentration as the independent variable. The ratio of mean number of fish in the center chamber during exposure to the total number of fish exposed (36) was the dependent variable.

## RESULTS

### Fathead Minnow

The 95% confidence intervals for the difference in numbers of fish among the three treatment periods during the first test series with nominal exposure concentrations of 3.0 and 6.0 mg/l total phenols indicated avoidance ($\nabla \leq 0.05$) of both toxicant concentrations. The second test series with exposure concentrations of 1.7 and 3.7 mg/l total phenols indicated significant avoidance at 3.7 mg/l total phenols. The third test series with exposure concentrations of 0.2 and 1.7 mg/l total phenols indicated that 1.7 mg/l was near the avoidance threshold. Fathead minnow showed no detectable response to concentrations of 0.7 mg/l total phenols.

Because fathead minnow preferred the center chamber in the absence of toxicant, avoidance was also defined as the relative reduction in utilization of the center as toxicant concentrations in the center increased. The ratio of mean number of fish utilizing the center chamber during the exposure period over the total number of fish tested (36) was plotted against calculated center concentrations (based on dilution and complete mixing) to provide a concentration-response curve (Figure 2). Excellent fit was obtained to the logistic model; model parameters were significant at $\nabla \leq 0.005$. The model was used to estimate the concentration where 50% of the fish avoided the center chamber (Median Avoidance Concentration or $AC_{50}$) and moved to the control chambers. The estimated $AC_{50}$ was 1.54 mg/l total phenols with a 95% confidence interval of $1.42 < AC_{50} < 1.65$.

### Rainbow Trout

Response of rainbow trout to coal liquid WSFs was different than that noted for fathead minnow. Although trout also preferred the center chamber during pre-exposure and post-exposure periods, test fish did not avoid the toxicant by moving into control chambers during exposure periods. At concentrations of 3.1 and 6.3 mg/l total phenols, rainbow trout remained in the center chamber (~3.1 mg/l) despite mortalities that exceeded 50% of the test group. At 2.4 and 4.6 mg/l total phenols, rainbow trout still preferred the center. However, at the lowest concentrations tested, significant ($\nabla < 0.05$) movement occurred from the center chamber (1.0 mg/l total phenols) to chambers with toxicant concentrations $\geq 2.2$ mg/l phenols. Overall, it appears that rainbow trout may be attracted to concentrations near 2.0 mg/l total phenols.

## DISCUSSION

Avoidance, or lack thereof, can be related to toxicity data for the same test material and species (Figures 3 and 4). The estimated $AC_{50}$ for fathead minnow of 1.5 mg/l estimated total phenols is about 24% of the 96 hr acute $LC_{50}$ based on total carbon (Becker et al. 1983). However, the observed avoidance threshold occurs at higher concentrations of total phenols than those known to cause sublethal or chronic effects such as inhibition of spawning and reduced juvenile growth (Dauble et al. 1983).

Although we observed no avoidance by rainbow trout of phenols in a complex-mixture WSF, De Graeve (1982) reported avoidance of 3.2-6.5 mg/l phenols tested as a

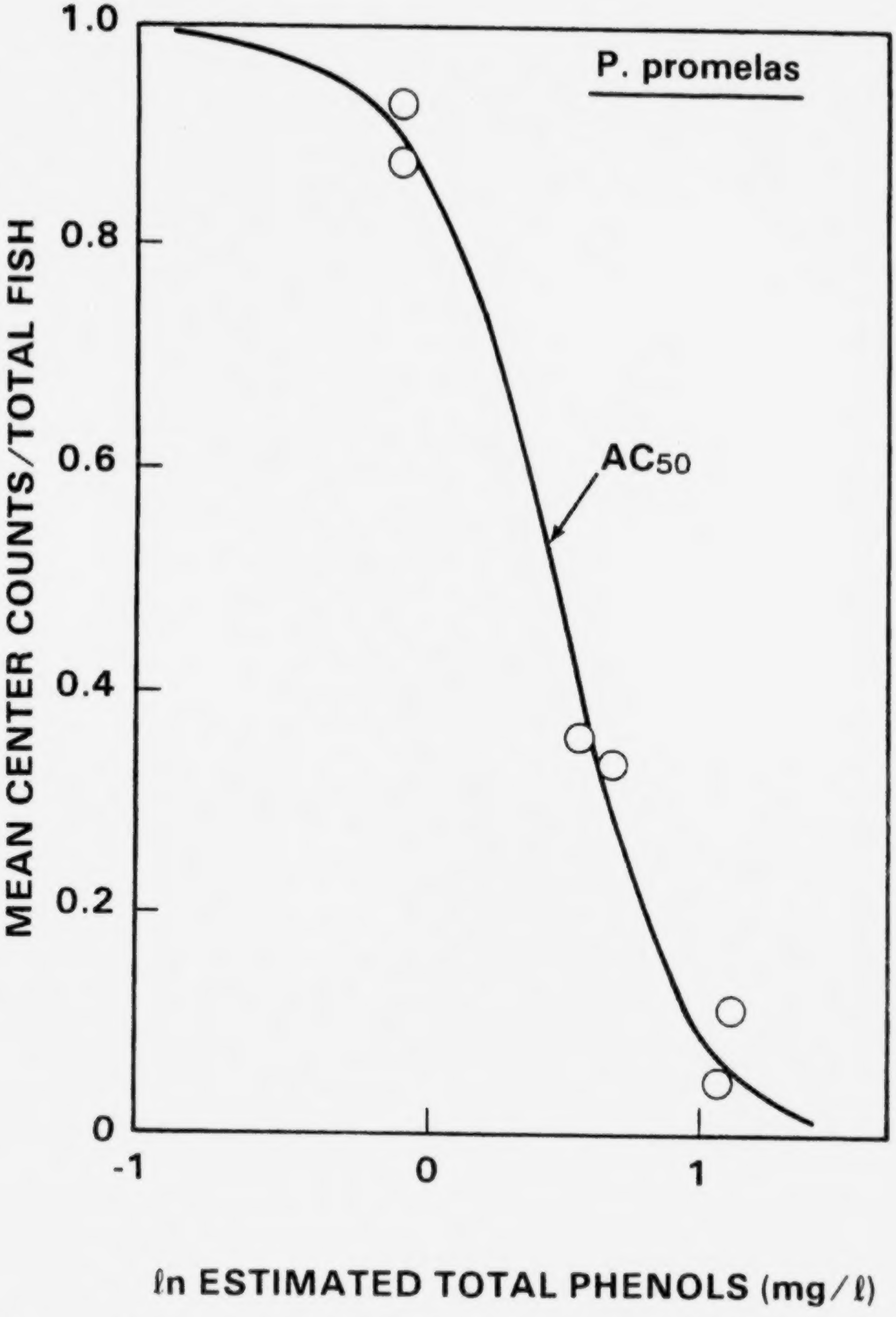

FIGURE 2 CONCENTRATION-RESPONSE CURVE FOR AVOIDANCE REACTION OF FATHEAD MINNOW EXPOSED TO A COAL LIQUID WATER SOLUBLE FRACTION (derived from Dauble et al. ms submitted)

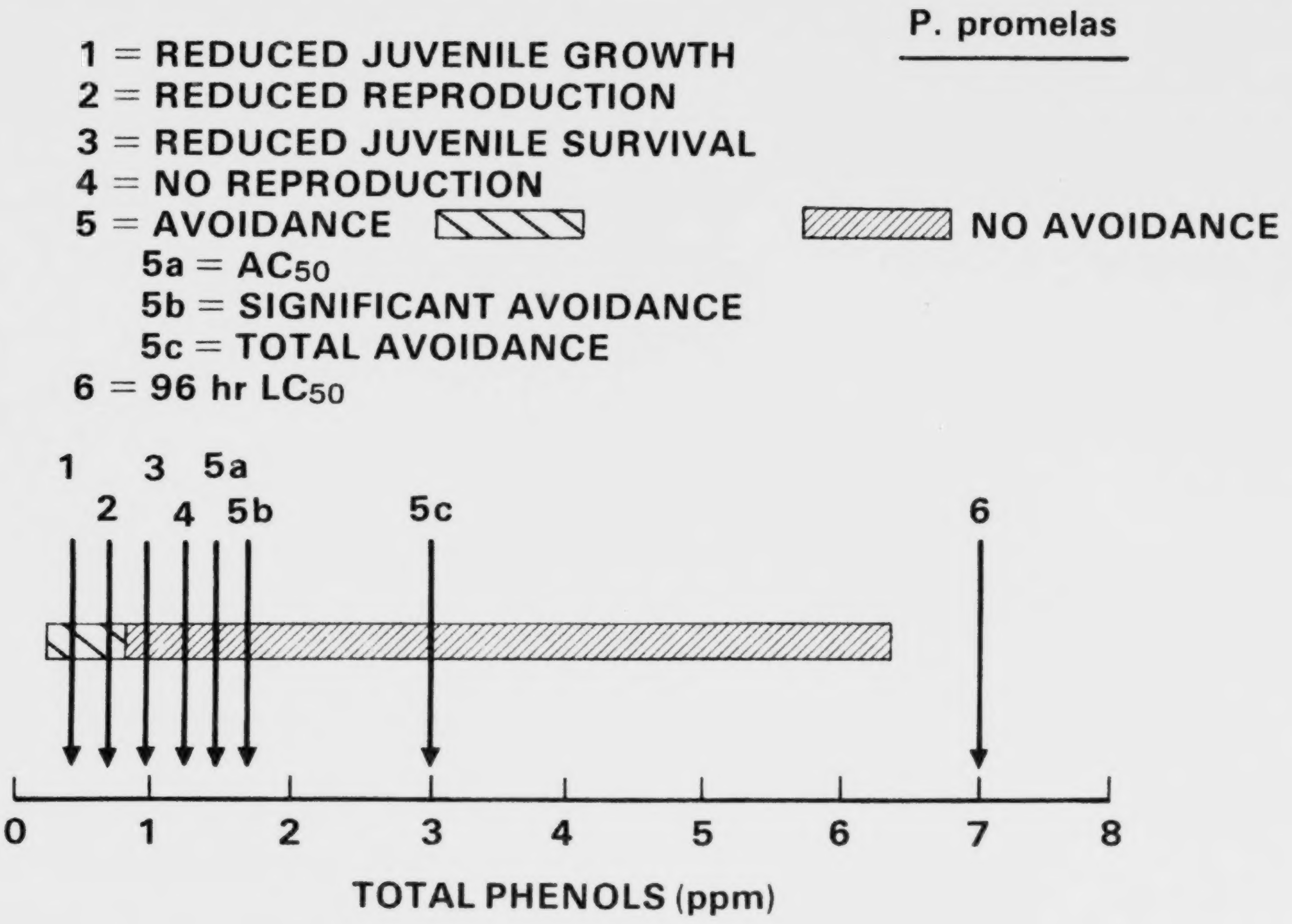

FIGURE 3 COMPARISON OF ACUTE AND CHRONIC TOXICITY AND AVOIDANCE DATA FOR FATHEAD MINNOW EXPOSED TO A COAL LIQUID WATER SOLUBLE FRACTION. ACUTE $LC_{50}$ CALCULATED FROM BECKER ET AL. (1983); CHRONIC EFFECTS DATA FROM DAUBLE ET AL. (1983)

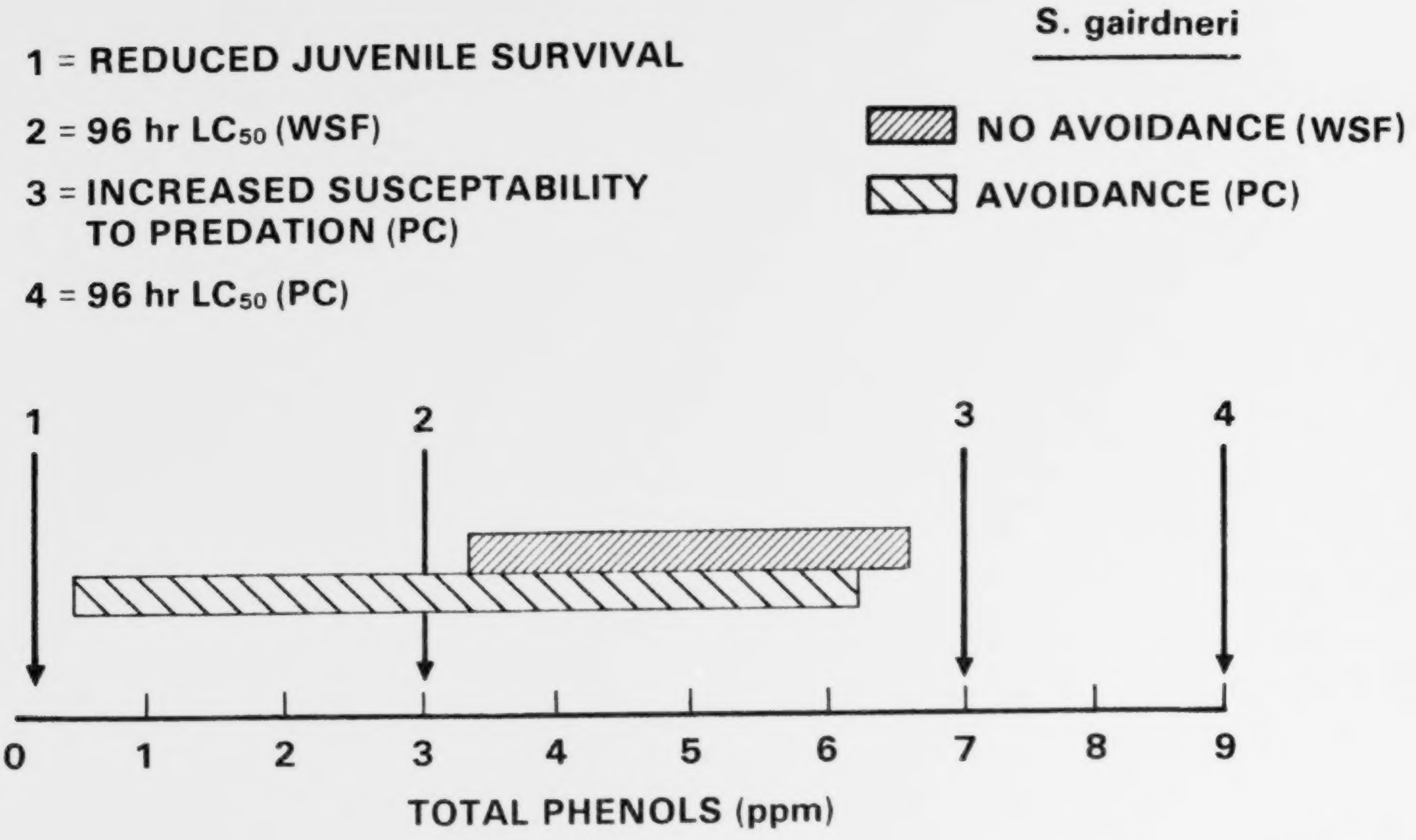

FIGURE 4 COMPARISON OF ACUTE AND CHRONIC TOXICITY AND PREDATION SUSCEPTABILITY AND AVOIDANCE DATA FOR RAINBOW TROUT EXPOSED TO A COAL LIQUID WATER SOLUBLE FRACTION (WSF) OR ITS CONSTITUENT PHENOLICS. ACUTE $LC_{50}$ FOR WSF CALCULATED FROM UNPUBLISHED DATA; CHRONIC EFFECTS DATA FOR WSF FROM DAUBLE ET AL. (1983); DATA ON SUSCEPTIBILITY TO PREDATION AFTER EXPOSURE TO PURE COMPOUND (PC) FROM SCHNEIDER ET AL. (1980); AVOIDANCE DATA FOR PC FROM DE GRAEVE (1982)

pure compound. Maynard and Weber (1981) showed that pre-smolt salmon avoided hydrocarbon components at lower concentrations when the hydrocarbons were presented individually than when combined in a simulated fuel mixture. Although our test procedures for evaluating avoidance differed from those of De Graeve (1982), results of pure compound studies may not reflect those of studies with complex mixtures. Toxicological responses may also differ. Acute $LC_{50}$ values obtained for rainbow trout with several individual phenolic compounds (De Graeve 1980), we generally higher than that observed for phenolics in our complex mixture (Figure 4). This phenomenon has also been shown in studies of mutagenicity, carcinogenicity and biouptake (Gray, in press).

Our failure to detect avoidance by rainbow trout of the coal liquid WSF in our test system may reflect the species inherent territoriality. Stevens et al. (1980) concluded that territorial behavior of rainbow trout influenced avoidance response to lethal concentrations of gas supersaturated water. Intraspecific behavior may establish territories in clean areas of the test chamber, thus, leaving only contaminated areas for subordinates.

## Future Needs

During the past several years, an extensive data base has been generated on the acute and chronic toxicity of coal liquid WSFs to aquatic organisms. The avoidance data for fathead minnow can be linked with the existing data base for complex mixtures in an initial predictive model that considers acute and chronic toxicity and behavioral avoidance.

Existing toxicological models, for example, the relationship between mortality and concentration and between avoidance and concentration (Figure 5a) may be used to develop a joint concentration/avoidance/mortality function shown conceptually in Figure 5b. The joint function suggests that some mortalities will occur at intermediate concentrations, while high survival may be anticipated at high (assuming avoidance occurs) and low concentrations. However, the initial joint function ignores several phenomena such as the relationship between concentration and time to death. If extreme high concentrations cause instantaneous mortality, there will be no avoidance of the toxicant and the concentration/avoidance/mortality function would be as shown conceptually in Figure 5c. Additionally, the influence of various environmental stimuli must be considered. For example, if the urge to spawn overrides the avoidance response, the joint function might look as indicated in Figure 5d.

Our results indicate that effects of hazardous materials on the behavior of fish may be expected to vary with species, natural schooling instincts, matrix of the toxic material, and other factors. Additionally, fish that avoid acutely lethal concentrations, may not detect or avoid concentrations causing longer-term population effects (growth, reproduction, etc.). Acute and chronic test results may only indicate problems at concentrations that are below the avoidance threshold for a given set of conditions and a given species. Models that link toxicological and behavioral data and consider the influence of environmental and other variables (feeding, schooling, spawning, etc.) on fish response are needed to allow more realistic assessments than can presently be attempted using acute and chronic toxicity data alone.

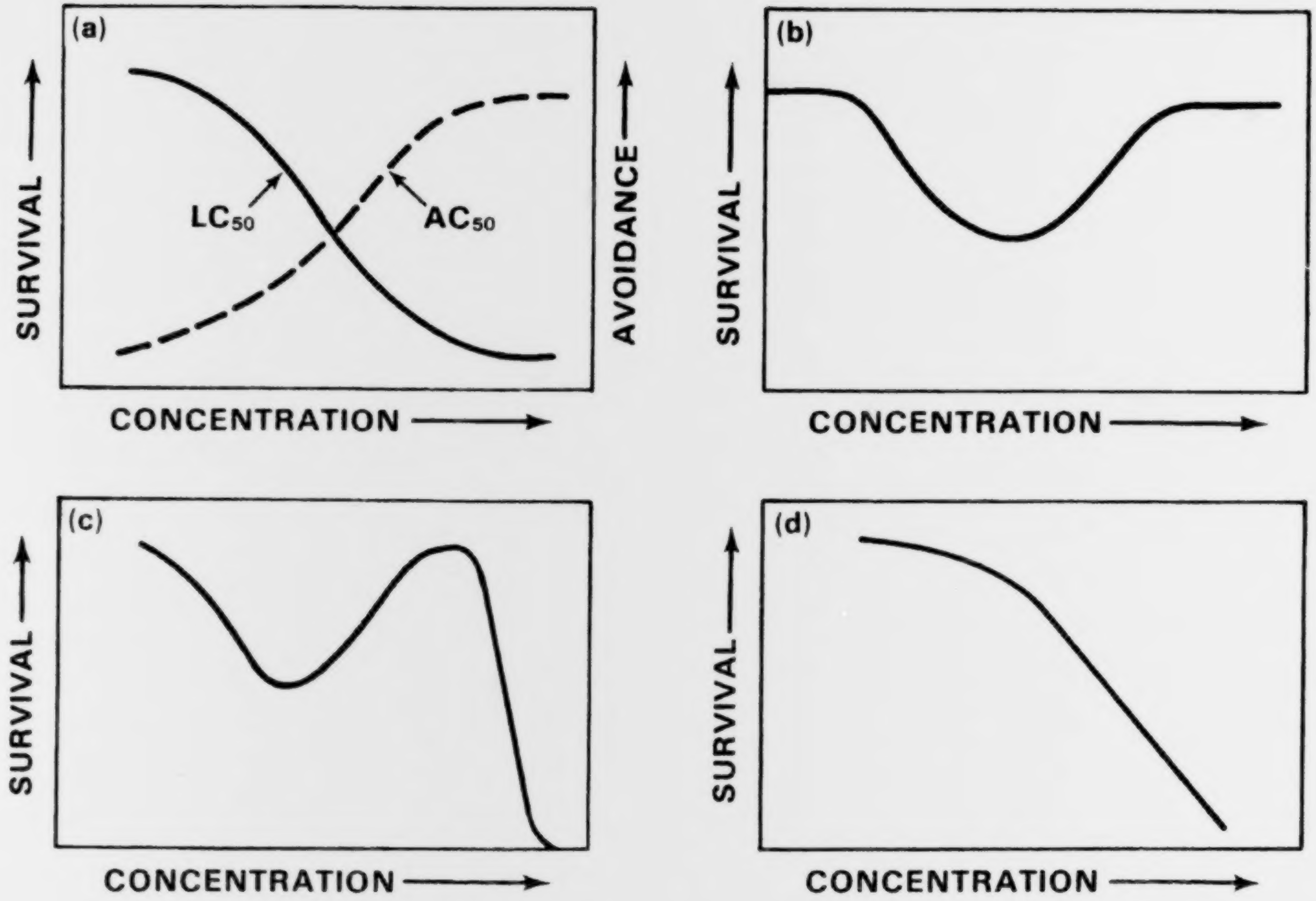

FIGURE 5 CONCEPTUAL MODELS: A) SEPARATE CONCENTRATION/AVOIDANCE AND CONCENTRATION/MORTALITY FUNCTIONS, B) JOINT CONCENTRATION/AVOIDANCE/MORTALITY FUNCTION, C) EFFECT OF URGE TO SPAWN ON JOINT FUNCTION, D) EFFECT OF INSTANTANEOUS MORTALITY ON JOINT FUNCTION

## ACKNOWLEDGEMENTS

We thank C. D. Becker and D. H. Fickeisen who critically reviewed the manuscript, E. W. Lusty who provided technical assistance in the laboratory, and the Pittsburg Midway Coal Mining Co., who provided the SRC-II blend. This study was supported by the U.S. Department of Energy (DOE), Ecological Research Division, Office of Health and Environmental Research under Contract DE-AC06-76RL0-1830 with Battelle Memorial Institute, Pacific Northwest Laboratories. Mention of tradenames in the manuscript does not imply endorsement by Battelle or DOE.

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## LIST OF FIGURES

Figure 1 Avoidance chamber (from Gray et al. 1983).

Figure 2 Concentration-response curve for avoidance reaction of fathead minnow exposed to a coal liquid water soluble fraction (derived from Dauble et al. ms submitted).

Figure 3 Comparison of acute and chronic toxicity and avoidance data for fathead minnow exposed to a coal liquid water soluble fraction. Acute $LC_{50}$ calculated from Becker et al. (1983); chronic effects data from Dauble et al. (1983).

Figure 4 Comparison of acute and chronic toxicity and predation susceptability and avoidance data for rainbow trout exposed to a coal liquid water soluble fraction (WSF) or its constituent phenolics. Acute $LC_{50}$ for WSF calculated from unpublished data; chronic effects data for WSF from Dauble et al. (1983); data on susceptibility to predation after exposure to pure compound (PC) from Schneider et al. (1980); avoidance data for PC from De Graeve (1982).

Figure 5 Conceptual models: a) separate concentration/avoidance and concentration/mortality functions, b) joint concentration/avoidance/mortality function, c) effect of urge to spawn on joint function, and d) effect of instantaneous mortality on joint function.

# HORMETIC EFFECTS IN TOXICANT AVOIDANCE RESPONSES

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HADJINICOLAOU, J. and G. LAROCHE. 1985. Hormetic effects in toxicant avoidance responses. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 337-344.

The emergence of behavioral toxicology is a relatively recent development and has received limited recognition prior to Weiss (1969). It is now increasingly acknowledged that behavioral responses to toxicant exposures may be critical for the survival of species and ecosystems. It may also provide an empirical basis for legislative regulations on acceptable concentrations of pollutants in the environment (Mello 1975).

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L'apparition de la toxicologie comportementale est un fait relativement récent, puisqu'avant Weiss (1969) cette discipline n'avait reçu qu'une reconnaissance limitée. Actuellement, on reconnaît de plus en plus que les réactions comportementales aux expositions toxiques peuvent être d'une importance cruciale pour la survie des espèces et des écosystèmes. Elles peuvent également fournir une base empirique pour l'établissement de règlements régissant les concentrations acceptables de polluants dans l'environnement (Mello 1975).

# INTRODUCTION

The emergence of behavioral toxicology is a relatively recent development and has received limited recognition prior to Weiss (1969). It is now increasingly acknowledged that behavioral responses to toxicant exposures may be critical for the survival of species and ecosystems. It may also provide an empirical basis for legislative regulations on acceptable concentrations of pollutants in the environment (Mello 1975).

It has been suggested that avoidance-preference reactions to a variety of toxins can be related to effects that pollutants may exert on chemoreceptors (Bardach et al 1965), mechanoreceptors (Gardner & LaRoche, 1973) or both. A morphological or biochemical lesion may remain dormant for long periods of time until it manifests itself in later life, in the form of behavioral or functional disorders (Nair 1968). Also, in 1969, Sprague inspired by the results of Hasler (1950), suggested that fish had their sensory perception dulled by phenol and p-chlorophenol.

Paracelcus in the 16th century wrote that many substances which are recognized toxic may be beneficial in small amounts (Stebbing, 1982). This phenomenon was formally identified by Schulz nearly 100 years ago (1888) in experiments with yeast.

Southam and Ehrlich in 1942 first proposed the term "hormesis" to describe "a stimulatory effect of subinhibitory concentrations of any toxic substance on any organism" and this definition is generally accepted. Specifically, hormesis is the name given to apparent or real stimulatory effects caused by low levels of potentially toxic agents. In this case, the suggestion that low doses produce harmful effects in proportion to dosage is invalid. Hormesis is a general phenomenon in which exposure of varied organisms to traces or low levels of many toxic substances or agents actually stimulate physiological mechanism in a manner that appears to benefit health and possibly survival (Luckey 1980). In recent years some interpretations have been given to stimulatory effects of low toxicant concentrations in a wide variety of organisms (Stebbing 1982).

In 1975 Luckey, reviewing the types of effects caused by pollutants and toxic agents, identified four types of concentration-response curves (Figure 1). The ∇-curve is the familiar pattern commonly observed for the effect of a toxic substance, showing no departure of the process or state from normal at low concentrations, followed by a progressive inhibitation above a threshold concentration. Curves β through δ show hormesis and describe responses most frequently observed when tests with low concentrations of various toxicants are analysed. Curve β shows a single stimulatory peak at concentrations immediately below those that become progressively inhibitory.

For the curves γ and δ, Luckey suggested that there are specific types of dose-response relationships and must remain questionable until more data are available. Generally, it is well documented that hormesis is a response that occurs in living organisms in the forms of a stimulation in biological activities at the cellular level (Stebbing 1982).

Using a 30 ft. long channel an avoidance apparatus was designed to obtain time-lapsed three dimensional analysis of fish positioning (Hadjinicolaou 1982, 1983). With this new apparatus effects of different toxicants (polymers, monomers, industrial effluents, D, etc.) were tested on rainbow trout. The results (i.e. Figures 2, 3, 4, 5) confirm that all four types of avoidance-preference dose-response curves of Luckey may be observed and

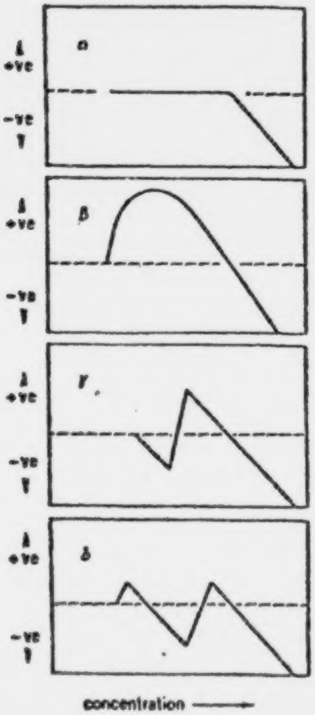

FIGURE 1 VARIOUS TYPES OF CONCENTRATION-RESPONSE CURVES IDENTIFIED BY LUCKEY (1975)

that at low concentrations some toxicants may attract rather than repel certain organisms. This response is interpreted as representing a behavioral extension of hormesis. It should be emphasized however that preference or attraction does suggest that beneficial effects will ensue.

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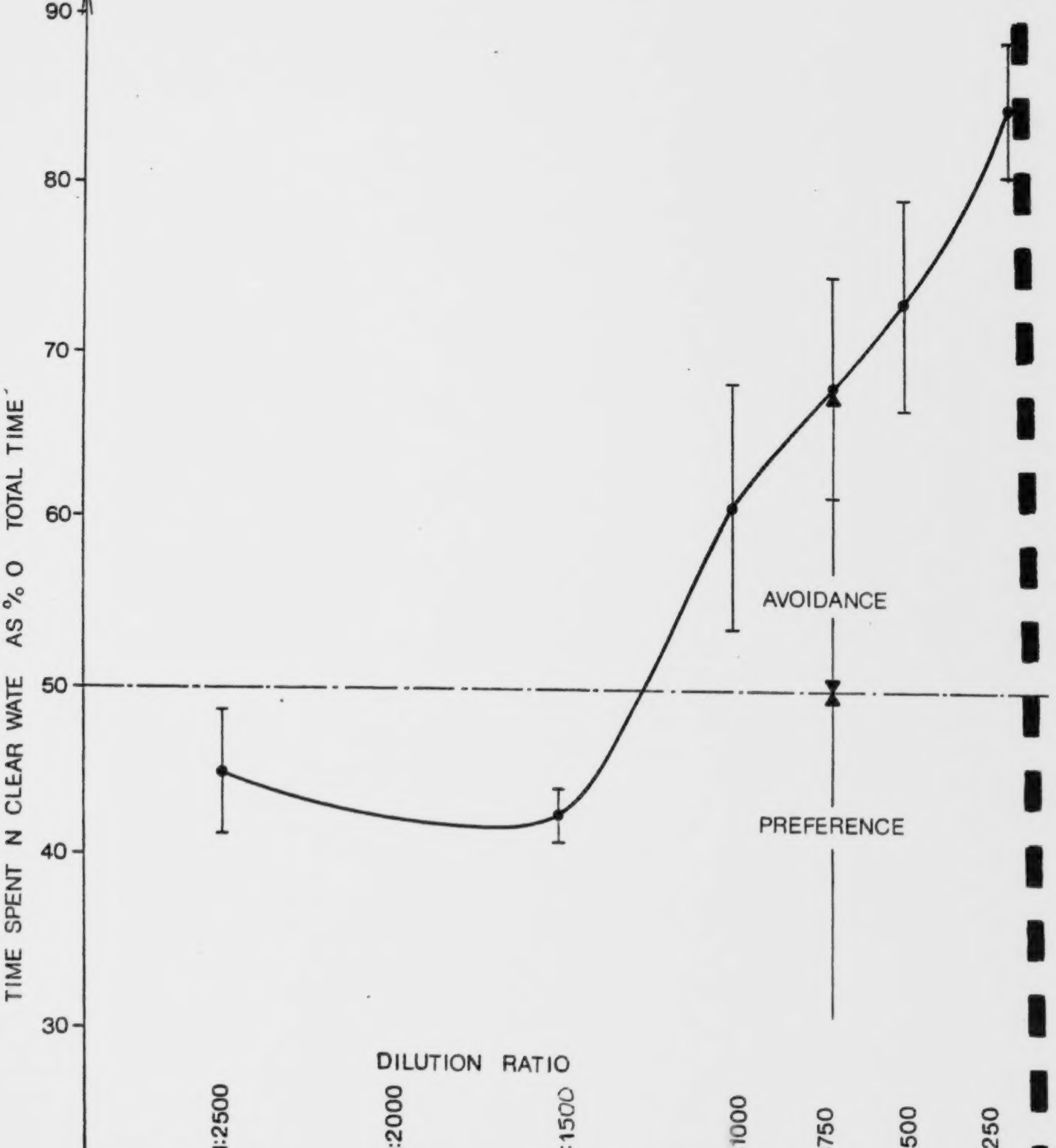

FIGURE 2 AVOIDANCE CURVE OF POLYMER A

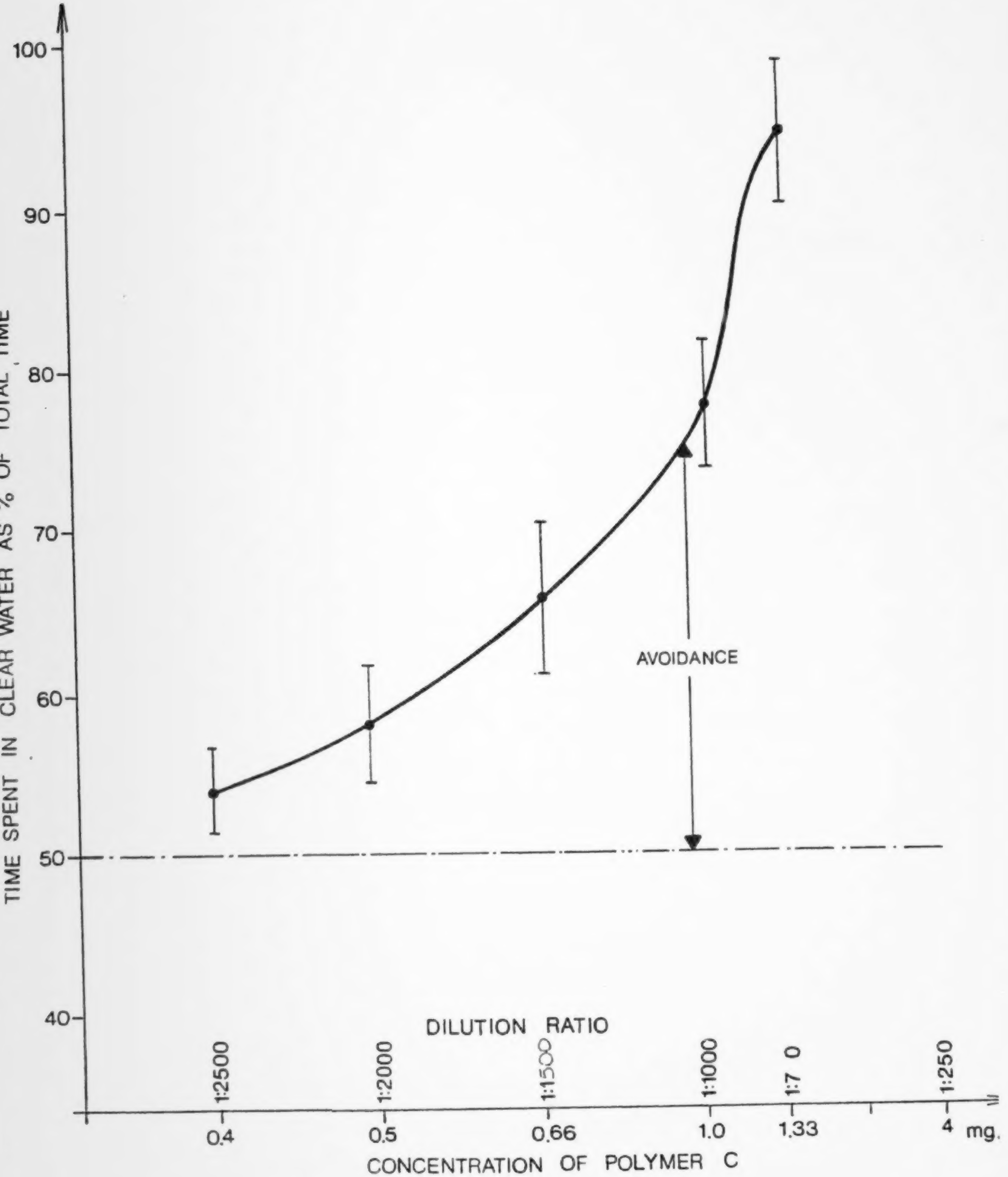

FIGURE 3 AVOIDANCE CURVE OF POLYMER C

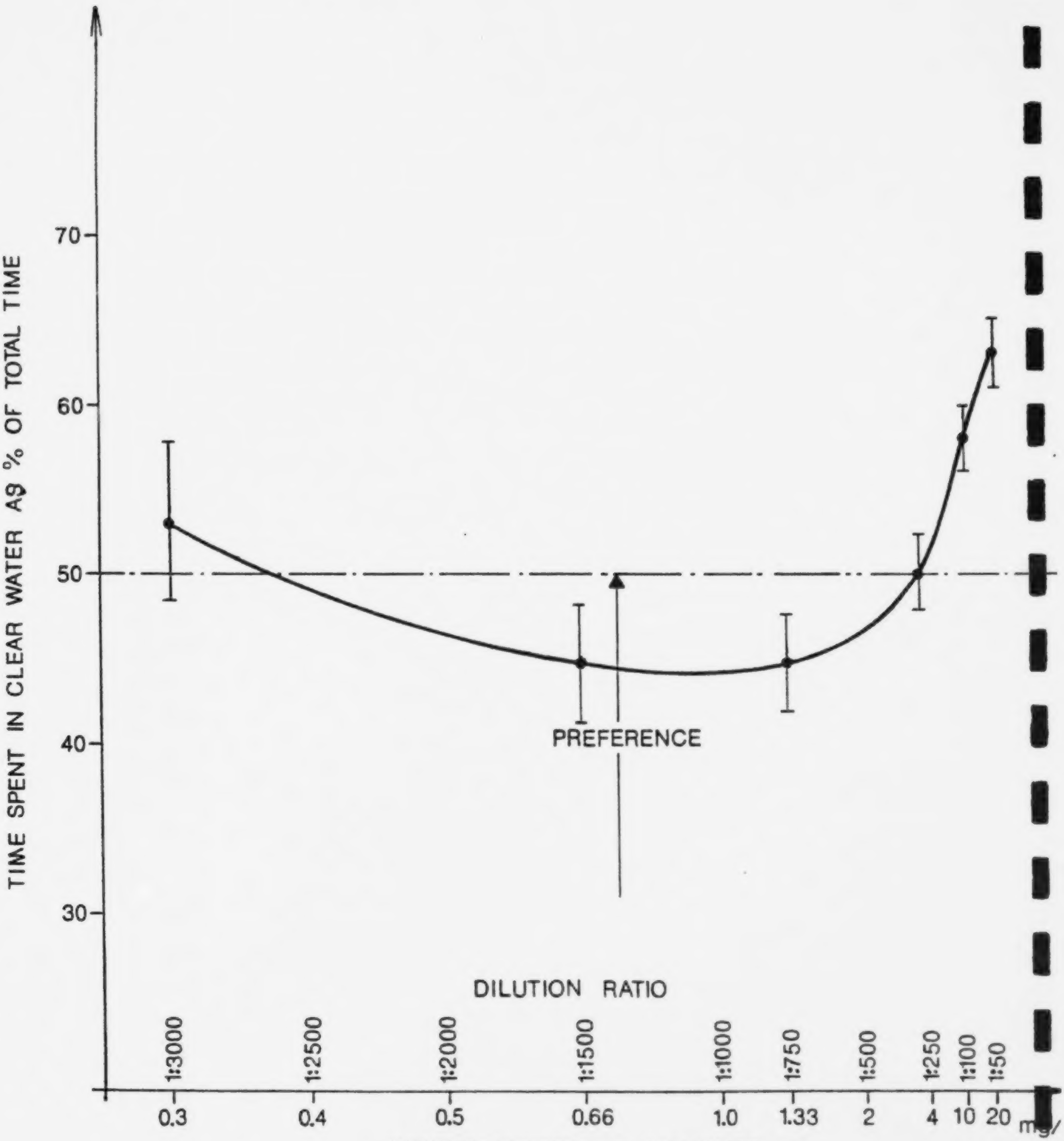

FIGURE 4 AVOIDANCE CURVE OF MONOMER A

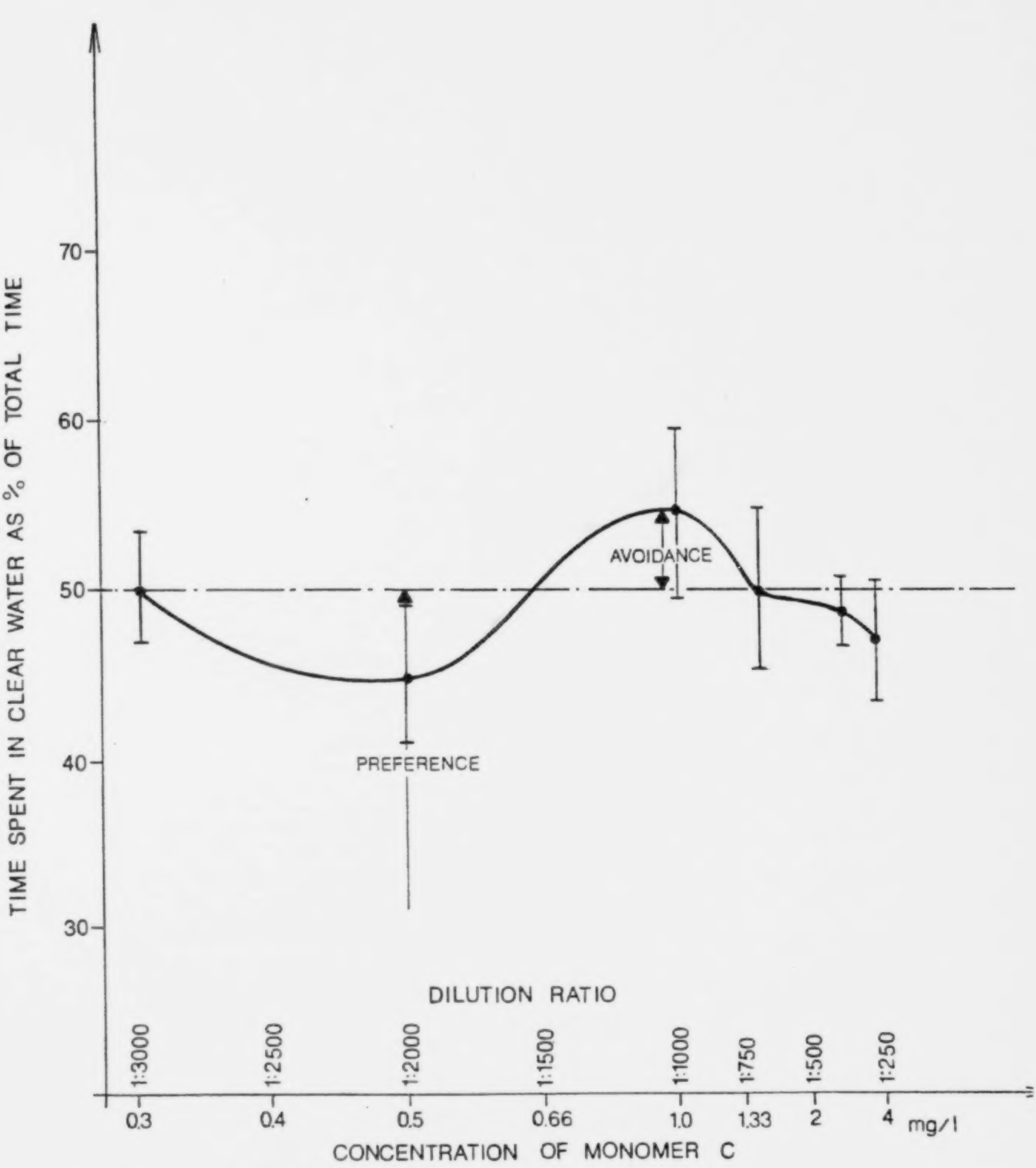

FIGURE 5 AVOIDANCE CURVE OF MONOMER C

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# ETHYL METHANESULPHONATE GENOTOXICITY IN BRACHYDANIO RERIO EMBRYOS

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SMITH, I.R., V.E. VALLI, G.R. CRAIG, D.A. ROKOSH, and H.F. FERGUSON. 1985. Ethyl methanesulphonate genotoxicity in Brachydanio rerio embryos. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 345-349.

Interest in the use of fish to assess the genotoxicity of chemicals sparked an investigation into the response of embryos to a solution of the recognized mutagen and carcinogen, Ethyl methanesulphonate (EMS). This approach has the advantage of avoiding chemical concentration of samples and measuring a response in a higher organism.

Brachydanio rerio (Zebrafish) embryos were exposed in 20 ml. of dechlorinated water immediately after fertilization for 24 hours to levels of 0,1,10,100 and 1000 mg/L EMS. Non-moribund embryos were examined grossly and as aceto-orceing squashes to determine the developmental stage, mitotic index (the total number of late anaphases), pyknotic index (number of pyknotic cells per 100 normal cells), micronuclei (small nuclei in yolk-sac cells) and the number and type of chromosome division aberrations visible in 20 late anaphases.

Controls were not significantly different and were pooled (n=40) yielding a mitotic index of 27.8, a pyknotic index of 0.68, micronuclei numbers of 0.8/100 and an anaphase aberration frequency of 0.75/20. Reduced growth was seen at 100 mg/L and above. EMS treated groups (n=10) exhibit no significant concentration related differences in mitotic index, but a significant increase in pyknotic cells was seen in 100 mg/L and above. Concentration related significant increases in anaphase aberration frequency were found at level of 1 mg/L EMS and above, however variability prevented a similar finding for micronuclei.

The concentration dependant increase in chromosome damage is indicative of genotoxity, and correlates with the activity of the test compound. Anaphase aberrations in Zebrafish embryos appear to be a sensitive indicator of waterborne genotoxins when compared to other studies utilizing adult fish and alternative genotoxicity endpoints.

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L'intérêt suscité par l'utilisation du poisson afin d'évaluer la génotoxicité des produits chimiques nous a amenés à faire une étude sur la réaction des embryons à une

solution d'un produit mutagène et cancérigène reconnu, l'éthyl méthanesulphonate (EMS). Cette méthode a l'avantage de permettre d'éviter la concentration chimique des échantillons et de mesurer la réaction chez un organisme supérieur.

Des embryons de dards-perches (Brachydanio rerio) ont été exposés dans 20 ml d'eau déchlorée, immédiatement après fécondation, pendant 24 heures à des niveaux de 0,1, 10, 100 et 1 000 mg/L de EMS. Les embryons non moribonds ont été examinés grossièrement et à l'état de broyat d'acéto-orcine, afin de déterminer le stade de développement, l'indice mitotique (nombre total d'anaphases tardives), l'indice pycnotique (nombre de cellules picnotique pour 100 cellules normales), les micronoyaux (petits noyaux dans les cellules du sac vitellin), le nombre et le type d'aberrations des divisions chromosomiques visible dans 20 anaphases terminales.

Les témoins n'étaient pas sensiblement différents et ils ont été regroupés (n = 40), présentant un index mitotique de 27,8, un indice pycnotique de 0,68, des nombres de micronoyaux de 0,8/100 et une fréquence d'aberrations d'anaphases de 0,75/20. Une réduction de la croissance a été observée à partir de 100 mg/L. L'indice mitotique chez les groupes traités à l'EMS (n = 10) ne présentait pas de différences de concentration significatives reliées à la concentration, mais une augmentation significative des cellules pycnotiques a été observée à des concentrations égales ou supérieures à 100 mg/L. Des augmentations importantes de la fréquence des aberrations d'anaphases, en fonction de la concentration, ont été observées au niveau de 1 mg/L d'EMS, et au-dessus, mais la variation a empêché de noter un résultat similaire pour les micronoyaux.

L'augmentation des altérations chromosomiques en fonction de la concentration est un indice de génotoxicité, et en est reliée à l'activité du produit d'essai. Les abérrations d'anaphases dans les embryons de dards-perches semblent être un indicateur sensible des génotoxines apportées par l'eau, comparé à d'autres études qui utilisent des poissons adultes et d'autres points limites de génotoxicité.

## EXTENDED ABSTRACT

Concern over waterborne hazardous contaminants has sparked many studies into their detection and possible health effects. An area receiving a great deal of interest is possible carcinogenicity and/or mutagenicity. Tumours have been found in fish exposed to polluted waters, and mutagenicity has been detected using the Ames Salmonella test in a variety of wastes and drinking waters (1).

The use of aquatic species to investigate mutagenicity (95% of mutagens are also carcinogens) has led to fish tissue and bile extracts being tested with the Ames test (1,2). The induction of chromosome damage has been detected in fish exposed to mutagens and polluted water. Two types of chromosome damage assays, the Micronucleus test (MN) and Anaphase abnormalities assay (AA) have been recently utilized in fish. As both test types require a rapidly dividing cell population, the use of fish embryos was suggested. This approach has the advantages of combining the relevance of an in-vivo whole organism assay with natural bioaccumulation and the inherent sensitivity of the embryo.

Zebrafish (Brachydanio rerio) were chosen to explore the use of embryos in mutagenicity testing because they produce a large number of eggs year-round, which are small, easy to expose in a static set-up, and develop rapidly. The response of these embryos to a proven mutagen/carcinogen was investigated to validate this approach. Ethyl methanesulphonate (EMS) was chosen because of the available data base in fish studies, its high water solubility and its proven activity. EMS is a direct-acting alkylating agent, in that it does not require enzymic activation to a reactive intermediate to react with DNA.

Newly fertilized embryos (2-64 cells) were exposed to 20 ml. of test solution in 50 mm. glass petri dishes in a water bath at 25°C. Dilution water was dechlorinated Toronto tap water, and embryos were exposed to 1, 10, 100 and 1000 mg/L EMS, with 4 control groups. Eggs were fixed after 24 hours (buffered formalin), dechloronated and treated for 15 minutes with 50% acetic acid, and then stained (5 minutes) and squashed in aceto-orcein containing 5% propionic acid.

The developmental stage of each of 10 embryos from each control and exposed groups was determined at fixation. Microscopically, the number of late anaphases provided a "mitotic index" and the number of dead (pyknotic) cells was determined. Micronuclei (small dense staining cytoplasmic bodies) were recorded in yolk-sac cells, and the first 20 late anaphases observed were examined for abnormalities. Late anaphase defects included lagging chromosomes (trailing whole or partial chromosomes), acentric fragments (small chromosome pieces without a centromere), attached fragments (with a centromere, attached to a division group by a thin thread of chromatin), chromatin bridges (thin strand of chromatin stretched between groups), multipolar figures (more than two sets of spindle fibers), and multiple damage (two or more indications of damage). Statistical analysis consisted of a students t-test ($P = 0.05$) and ANOVA.

EMS caused no significant mortality, though increased fungal growth was evident and caused limited mortality in some concentrations. Survival in the 1000 mg/L treated group was > 85% after 24 hours, however this chemical level was completely lethal if extended to 72 hours.

A reduction in developmental stage was present in both 1000 and 100 mg/L relative to control embryos, and wasn't recovered when embryos were removed to clean water after exposure for 24 hours. These embryos apparently developed normally, however all died immediately after hatching. 100 mg/L EMS had no similar effects.

No significant reduction in mitotic index was in evidence, due in part to wide inter-individual variability. The average number of late anaphases was 27.8 (n=40) in controls.

The number of pyknotic cells were significantly higher than control (0.68/100, n=40) in 1000 and 100 mg/L EMS, with values of 175/100 and 3.21/100 respectively. An insignificant increase in 10 and 1 mg/L was also seen.

Control micronuclei levels of 0.8/100 average featured wide variability, preventing the increase in 1000 and 100 mg/L to 4.5/100 and 2.2/100 respectively from being significant.

Control anaphase abnormality frequency averaged 0.75/20 (n=40). A significant increase was seen in 1000, 100 and 10 mg/L (levels of 14.7/20, 7.3/20 and 2.1/20 respectively) versus individual paired (n=10) controls. Low control variability facilitated their pooling (n=40), making the increase to 1.5/20 in 1 mg/L significant. AA frequency was significantly ($P < 0.001$, $r^2$ 78.7) related to the log of the concentration.

Four types of damage predominated in this study; attached and acentric fragments (indicative of breakage), bridges (incomplete translocations) and lagging chromosomes (possibly a toxicity induced "stickiness") accounting for 94% of the lesions seen in 1000 mg/L EMS. The relative increase above controls was greatest in lagging chromosomes, followed by both fragment types and distantly by bridges.

The growth reduction(developmental stage) evident was obviously due to cell death (pyknotics) rather than a reduced rate of division (mitotic index) or metabolism. The finding of cell death in a mitotic tissue may be a characteristic of mutagens, as their action is exerted upon the DNA during replication and/or duplication/repair processes. The effect of non-mutagenic chemicals on cell death in embryos must be assessed prior to confidence in "mitotic pyknosis" being an indicator of mutagenesis. The persistence of pyknotic cells may make them a valuable indicator, and indeed pyknosis in embryos induced by Benzo-a-pyrene has been reported (3).

Micronuclei are also quite persistant, providing a cumulative summation of damage, perhaps leading in part to the wide variability evident. The relationship of both MN and pyknotics to apoptosis (natural cell death being followed by the phagocytosis of nuclear material) must be studied better, however MN have been reported in adult fish exposed to EMS utilizing peripheral red-blood cells (4).

The most valuable indicator of chromosome damage was AA's. Providing a moment-in-time measure of genetic damage, many of the lesions seen can lead to micronuclei (lagging chromosomes and fragments) or cell death (possibly the majority of the defects observed). The sensitivity of this approach is comparable to or better that those using adult fish and other measures of genotoxicity. The finding of a concentration response over three orders of magnitude led to damage in up to 75% of anaphases observed, ranging upward from a control level of 4%. EMS induced a significant increase in genetic damage at a level < 1.5% of the $LC_{50}$, comparable to the application factor normally utilized to protect aquatic life.

Advantages of this approach are many, including its sensitivity. Classical approaches to genotoxicity require a low number of large chromosomes; fish with this characteristic are rare. The AA analysis utilized in this study can be applied to any mitotic tissue in any organism. Classical techniques measure damage to individual chromosomes, while in the AA approach, damage is visible to individual chromosomes at a lower magnification, increasing analysis speed. Colchicine (a spindle poison to accumulate metaphases) is essential in classical studies; its absence in this approach is a benefit as colchicine is mutagenic.

The use of AA'S (and MN) should be applicable to both laboratory and field work, for both in-situ exposures and resident fish sampling. This will facilitate the detection of mutagens in the environment, and their characterization under controlled laboratory conditions with the same basic approach. This study has validated that Zebrafish embryos respond in a number of ways to the direct acting mutagen Ethyl methanesulphonate. The best measure of genotoxicity was anaphase abnormalities, and this approach has numerous advantages over more classical approaches, with equal or better sensitivity.

## ACKNOWLEDGEMENTS

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# MARINE BIOASSAYS WITH LOBSTER LARVAE AND GAMMARIDS

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WOOD, C.S., J. DUNCAN, and K. WHEELAND. 1985. Marine bioassays with lobster larvae and gammarids. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 351-372.

To examine the toxicity of a diammonium phosphate fertilizer plant effluent discharging to the Baie des Chaleurs, a bioassay methodology incorporating lobster larvae (Homarus americanus) and gammarids (Gammarus oceanicus) was developed in consultation with the Canadian Department of Fisheries and Oceans. The gammarids were collected locally while the stage four lobster larvae were reared from gravid adult females. The lobsters were captured well away from the plant operations and transferred to individual hatcheries. After hatching, the first stage larvae remained near the surface, floated into the larval catchboxes and were subsequently transferred to Hughes rearing tanks. Larvae were maintained on a brine shrimp diet. A survival rate of 11-12.5% to stage four was observed. Gammarid stocks were maintained in flowing water throughout the bioassay period.

Standard 96-h static bioassays were conducted in an on-site laboratory to determine the $LC_{50}$ of both whole and decanted effluent.

Plant effluent was found to be toxic to gammarids in the concentration range of about 4-10%, and to lobster larvae in the range of 8-11%. Decant effluent was slightly less toxic than unsettled effluent and storage of effluent over a five-week period had no apparent effect on its toxicity.

The above results indicate that lobster larvae were slightly less sensitive than gammarids, and that both organisms were affected by the effluent pH, suspended solids and elements. However, the relative roles and importance of these parameters could not be quantified or estimated on the basis of the present work.

WOOD, C.S., J. DUNCAN, and K. WHEELAND. 1985. Marine bioassays with lobster larvae and gammarids. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 351-372.

Afin d'examiner la toxicité de l'effluent d'une usine d'engrais, à base de phosphate de diammonium, se déversant dans la baie des Chaleurs, nous avons mis au point une méthode de dosage biologique pour les larves de homards (Homarus americanus) et les gammarides (Gammarus oceanicus), en consultation avec le ministère canadien des Pêches et des Océans. Les gammarides ont été recueillis localement, tandis que les larves de homards de quatrième stade étaient élevées à partir de femelles gravides adultes. Les homards ont été capturés à bonne distance de l'usine, et transférés dans des centres d'élevage

individuels. Après incubation, les larves de premier stade sont restées près de la surface, ont flotté à l'intérieur des casiers de capture des larves et ont été transportées par la suite dans des bassins d'élevage Hughes. Elles ont ensuite été entretenues grâce à un régime à base d'artémia. Un taux de survie de 11 à 12,5 % au quatrième stade a été observé. Les stocks de gammarides ont été maintenus dans de l'eau courante tout au long de la période de bio-essai.

Des bio-essais standard sur 96 heures ont été réalisés dans un laboratoire sur les lieux afin de déterminer la CL50 de l'effluent à l'état brut et décanté.

On a noté que l'effluent de l'usine était toxique pour les gammarides dans une échelle de concentrations variant entre 4 et 10 %, et pour les larves de homards, entre 8 et 11 %. L'effluent décanté s'est montré légèrement moins toxique que l'effluent non déposé et le stockage de l'effluent sur une période de cinq semaines n'a pas eu d'effet apparent sur sa toxicité.

Ces résultats indiquent que les larves de homards étaient légèrement moins sensibles que les gammarides, et que les deux organismes ont été pertubés par le pH de l'effluent, les solides et les éléments en suspension. Cependant les rôles relatifs et l'importance de ces paramètres n'ont pu être quantifiés ni estimés dans les conditions de notre étude.

## INTRODUCTION

This presentation describes the development and application of a marine bioassay methodology for evaluating the toxicity of a phosphate fertilizer plant effluent.

1. A brief background is provided regarding:
    - the phosphate fertilizer process;
    - the effluent characteristics;
    - the history of environmental monitoring at this site;
    - the selection of bioassay organisms and the development or adaptation of procedures.
2. The experimental details are then described including:
    - laboratory layout and equipment;
    - collection and acclimation methods;
    - bioassay methodology;
    - test materials.
3. The results are then presented and discussed for each organism and in terms of the main effluent characteristics.
4. Finally, overall conclusions are presented.

### Background

### Belledune Fertilizer Operations and Effluents

The Belledune Fertilizer Division of Noranda Mines Limited is located at Belledune, New Brunswick, on the Baie des Chaleurs (Figure 1). The plant is designed to produce 300 000 tonnes per year of diammonium phosphate (DAP), utilizing sulphuric acid, phosphate rock and liquid ammonia. The key process steps are as follow:

- The phosphate rock is reacted with sulphuric acid to form phosphoric acid and calcium sulphate or gypsum.

- The phosphoric acid is then filtered, concentrated and reacted with gaseous ammonia to form DAP.

- The gases from the rock acidulation are scrubbed with sea water which is then used to transport the by-product gypsum to the sea.

The effluent contains about 4% of suspended gypsum. The liquid portion is acidic and contains contaminants from the phosphate rock (P, F, metals, ...).

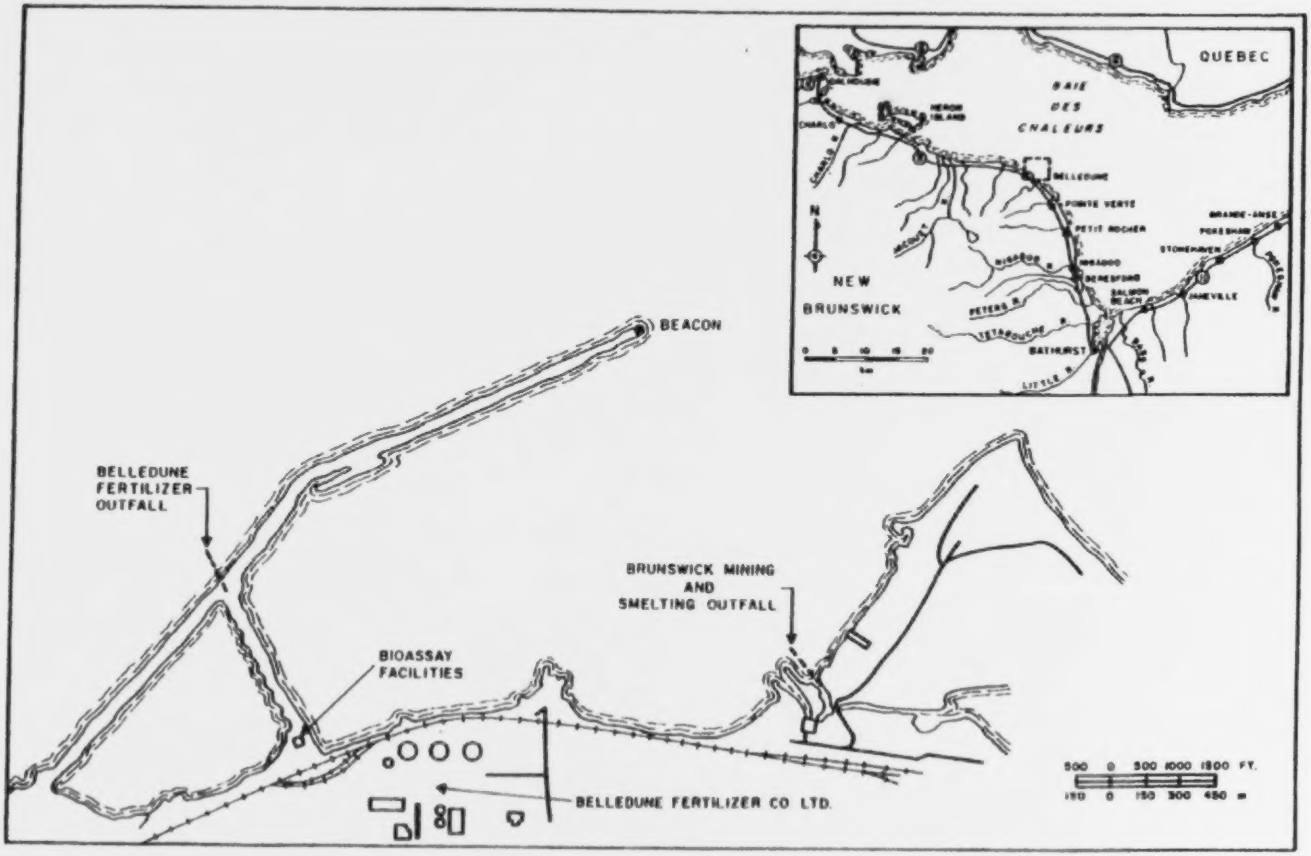

QUEBEC
BAIE DES CHALEURS
DALHOUSIE
HERON ISLAND
CHARLO
CHARLO R.
BELLEDUNE
POINTE VERTE
PETIT ROCHER
NIGADOO
BERESFORD
SALMON BEACH
BATHURST
GRANDE-ANSE
POKESHAW
STONEHAVEN
JANEVILLE
JACQUET R.
NIGADOO R.
PETERS R.
TETAGOUCHE R.
LITTLE R.
N
NEW BRUNSWICK
0 5 10 15 20
km
BEACON
BELLEDUNE FERTILIZER OUTFALL
BIOASSAY FACILITIES
BRUNSWICK MINING AND SMELTING OUTFALL
BELLEDUNE FERTILIZER CO LTD.
500 0 500 1000 1500 FT.
150 0 150 300 450 m

FIGURE 1 LOCATION OF THE ON-SITE BIOASSAY FACILITIES AT BELLEDUNE FERTILIZER, BELLEDUNE, NEW BRUNSWICK, 1980

### Marine Environmental Monitoring, 1967 to Present

A pre-operational benthic survey was conducted in 1967 and post-operational benthic surveys have been continued at intervals. The gypsum deposition is also monitored, sampled and mapped routinely. Other studies to evaluate the mixing zone include monitoring of fluoride levels in native marine organisms along with analyses of water samples.

### Bioassays

In 1979, the Noranda Research Centre, at the request of Belledune Fertilizer (BF), initiated a detailed study in co-operation with BF to delineate the outfall mixing zone in the vicinity of its plant operations at Belledune, New Brunswick, by means of static bioassays with indigenous marine species. During the first phase of on-site bioassays, preliminary toxicity results were obtained, and methodologies were developed and evaluated.

In early 1980, methodologies(1) and bioassay facilities were reviewed and finalized, in consultation with plant and Federal Department of Fisheries and Oceans (DFO) personnel. From May to August, a total of thirty-four 96-hour unaerated static bioassays were conducted on-site with the assistance of BF personnel, using (as in 1979) Gammarus oceanicus (scuds) and stage 4 Homarus americanus (lobster) larvae. Tests were conducted with several forms of plant effluent, as well as phosphoric and hydrochloric acid (effect of pH).

This report details the final methodologies and bioassay facilities, experimental work, test results and conclusions for the study. Figure 1 indicates the location of the bioassay facilities at the BF plant.

## 1980 EXPERIMENTAL PROGRAM

### Laboratory Facilities

The general layout of the laboratory facilities, which were housed in a wooden structure north of the BF operations, is shown in Figure 2. The major equipment items are described below.

a) A lobster hatchery consisting of six separate sections for the berried females, each connected to the larval section by a notched partition at which a larval catcher was positioned. Drain holes were added to permit cleaning of individual sections. Lagoon water was fed to each adult section and flowed over the notch to a standpipe in the larval section (Figure 3).

b) Eight commercial Hughes* larvae rearing tanks, with water circulation and overflow mechanisms (Figure 4).

c) Two gammarid holding tanks, consisting of five-gallon plastic pails with central screened overflow pipes, supplied with lagoon water and aeration.

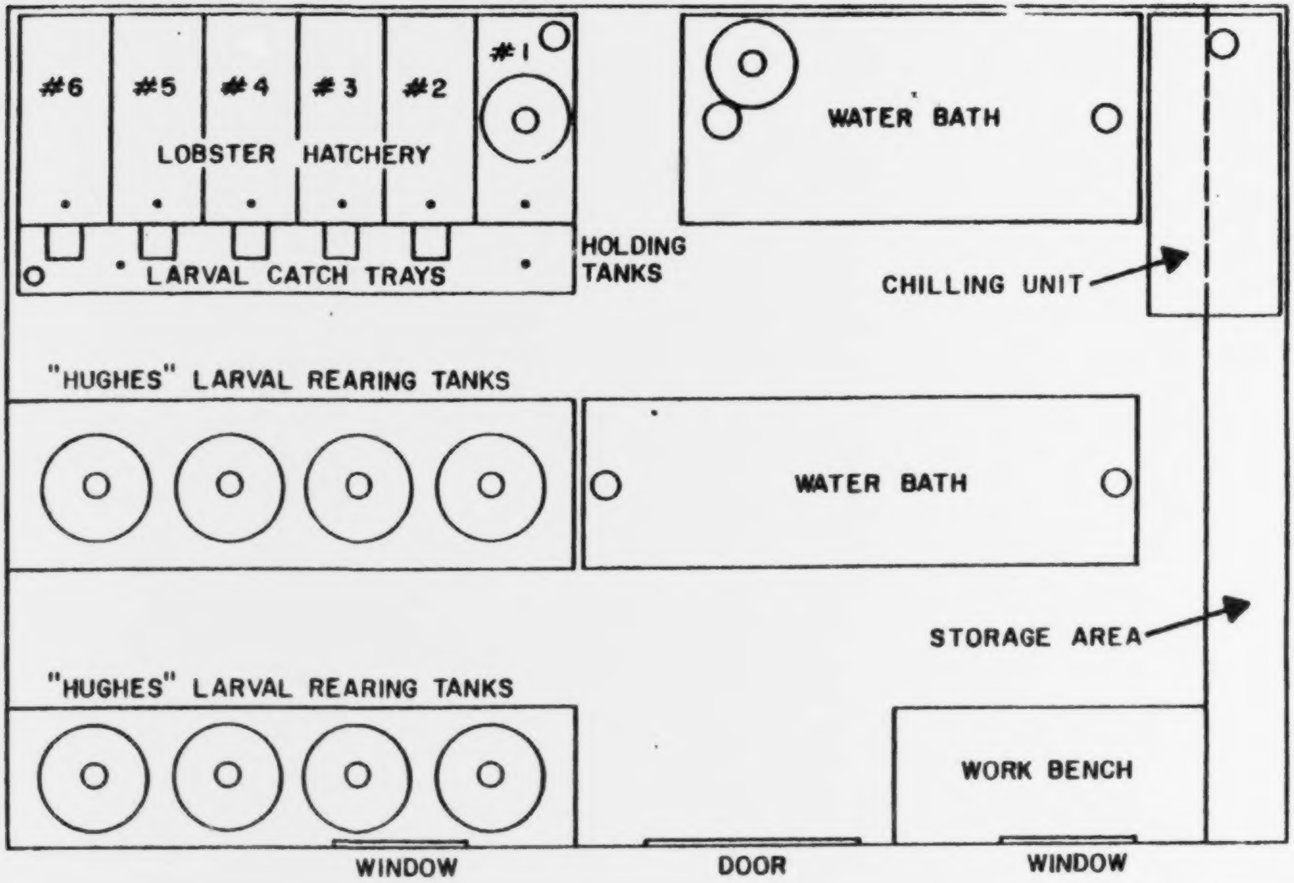
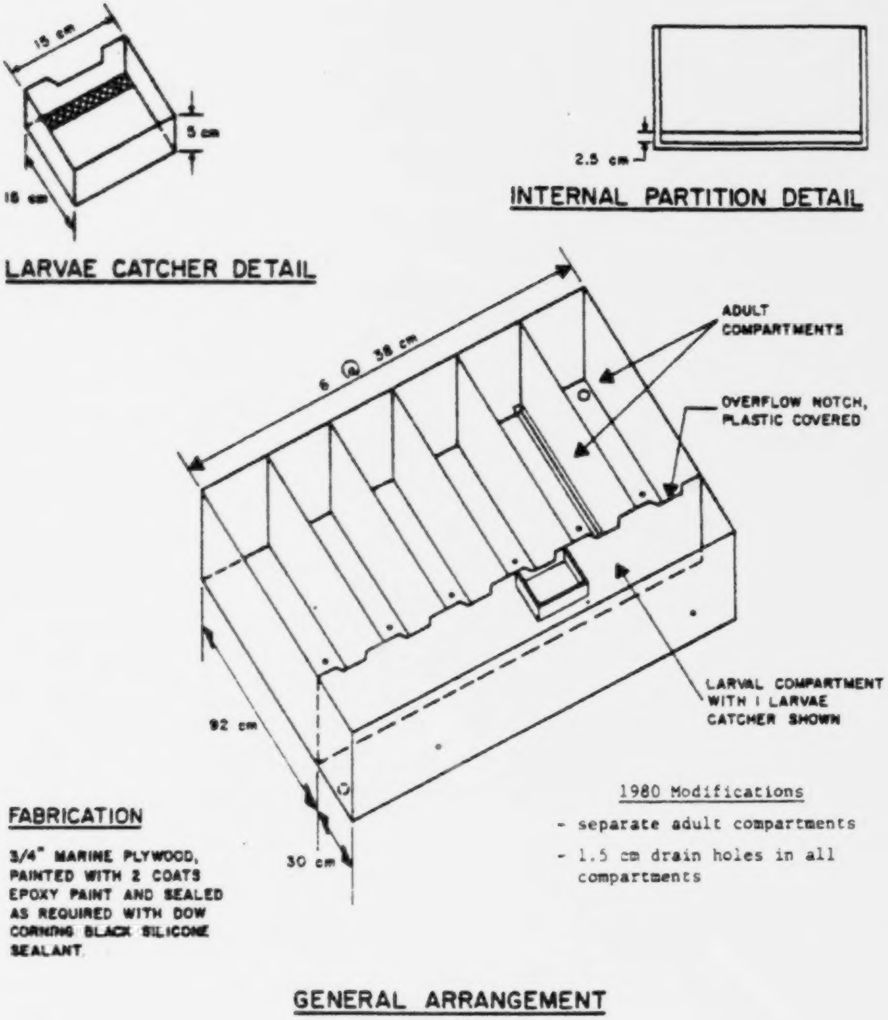

#6
#5
#4
#3
#2
#1
LOBSTER HATCHERY
HOLDING TANKS
LARVAL CATCH TRAYS
WATER BATH
CHILLING UNIT
"HUGHES" LARVAL REARING TANKS
WATER BATH
STORAGE AREA
"HUGHES" LARVAL REARING TANKS
WORK BENCH
WINDOW
DOOR
WINDOW

FIGURE 2 MODIFIED LAYOUT OF THE BELLEDUNE FERTILIZER BIOASSAY FACILITIES (1980)


15 cm
5 cm
16 cm
LARVAE CATCHER DETAIL
2.5 cm
INTERNAL PARTITION DETAIL
6 @ 38 cm
ADULT COMPARTMENTS
OVERFLOW NOTCH, PLASTIC COVERED
LARVAL COMPARTMENT WITH 1 LARVAE CATCHER SHOWN
92 cm
30 cm
GENERAL ARRANGEMENT

**FABRICATION**

3/4" MARINE PLYWOOD, PAINTED WITH 2 COATS EPOXY PAINT AND SEALED AS REQUIRED WITH DOW CORNING BLACK SILICONE SEALANT.

1980 Modifications

- separate adult compartments
- 1.5 cm drain holes in all compartments

FIGURE 3 MODIFIED ARRANGEMENT AND DETAILS OF THE LOBSTER LARVAE HATCHERY (1980)

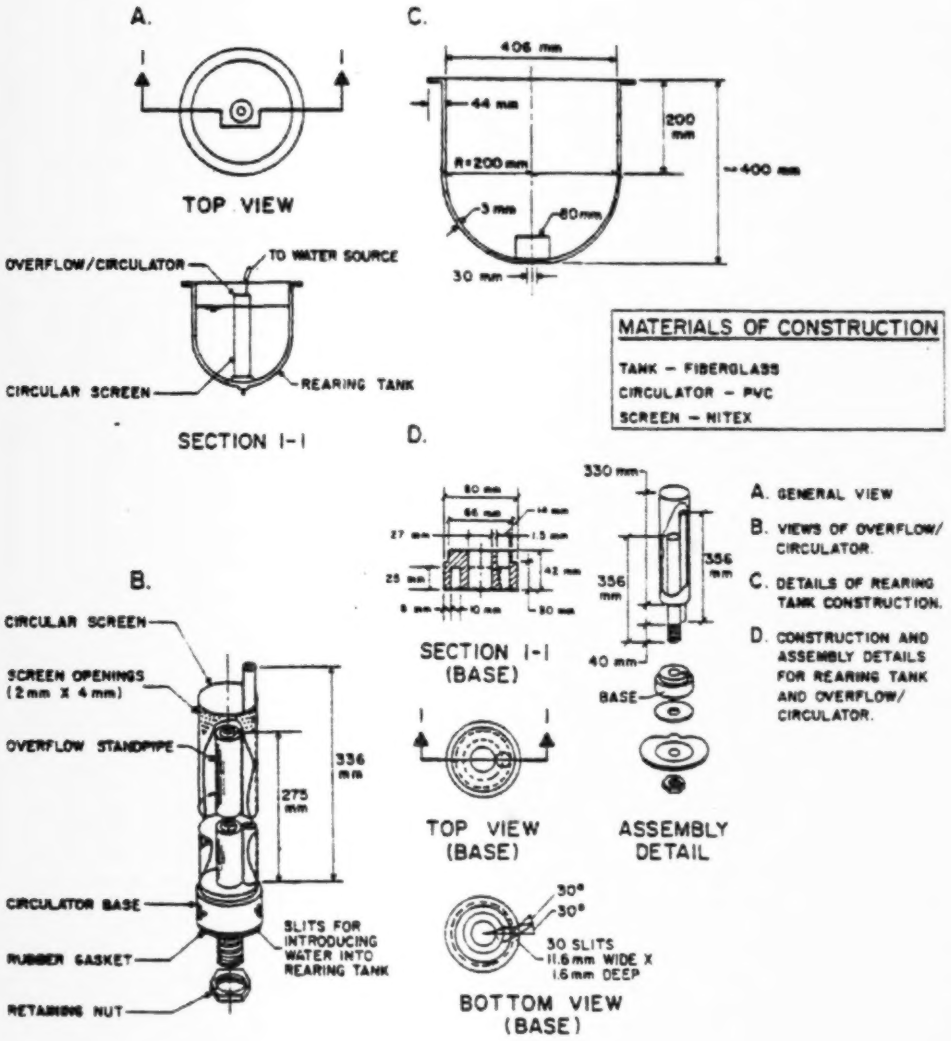

A.
I
I
TOP VIEW
OVERFLOW/CIRCULATOR
TO WATER SOURCE
CIRCULAR SCREEN
REARING TANK
SECTION I-I
C.
406 mm
44 mm
200 mm
R=200 mm
~400 mm
3 mm
80 mm
30 mm
MATERIALS OF CONSTRUCTION
TANK – FIBERGLASS
CIRCULATOR – PVC
SCREEN – NITEX
D.
80 mm
66 mm
14 mm
27 mm
1.5 mm
25 mm
42 mm
8 mm
10 mm
30 mm
SECTION I-I (BASE)
330 mm
356 mm
356 mm
40 mm
BASE
I
I
TOP VIEW (BASE)
ASSEMBLY DETAIL
30°
30°
30 SLITS 11.6 mm WIDE X 1.6 mm DEEP
BOTTOM VIEW (BASE)
B.
CIRCULAR SCREEN
SCREEN OPENINGS (2 mm X 4 mm)
OVERFLOW STANDPIPE
336 mm
275 mm
CIRCULATOR BASE
RUBBER GASKET
SLITS FOR INTRODUCING WATER INTO REARING TANK
RETAINING NUT
A. GENERAL VIEW
B. VIEWS OF OVERFLOW/CIRCULATOR.
C. DETAILS OF REARING TANK CONSTRUCTION.
D. CONSTRUCTION AND ASSEMBLY DETAILS FOR REARING TANK AND OVERFLOW/CIRCULATOR.

FIGURE 4 HUGHES LOBSTER LARVAE REARING TANK

d) Two bioassay assemblies, each consisting of a water bath into which the bioassay test vessels were placed. The baths consisted of 61 cm x 244 cm x 30.5 cm high open top plywood boxes, similar in construction to the lobster hatchery.

Salt water from the adjacent lagoon was supplied, via two interchangeable brass-fitted steel pumps and ABS plastic piping, at a maximum rate of about 100 L/min. Lighting was provided by two central 60-watt fluorescent fixtures on a 14 h on/10 h off cycle. Air was provided by two aquarium pumps; distribution was via tygon tubing, plastic tees, and Hagen airstones to the lobster hatchery and gammarid holding tanks.

## Collection and Acclimatization Methods

**Gammarids.** Gammarus oceanicus (gammarids or scuds) were collected as required from the intertidal vegetation near Pointe-Verte located approximately 8 km to the southeast of the fertilizer plant. The organisms were placed into seawater inside a five-gallon plastic pail with some marine vegetation, and immediately transported to the holding vessels in the bioassay laboratory. Precaution was observed to maintain the different collection stocks segregated from each other. A less than 1° C temperature difference existed between collection site water and holding water (lagoon).

Water flow rate was maintained to ensure at least 90% replacement every 2-3 hours, and dissolved oxygen levels were kept at 7-9 mg/L by aeration. The gammarids were not fed as the nutrients supplied in the lagoon water along with the marine vegetation in the holding tanks provided ample food sources.

Since the gammarids were being held in the lagoon water which also served as bioassay dilution water, no additional acclimation was necessary. The test organisms were held a minimum of six days prior to being tested.

Throughout the holding periods there was never any indication of disease, visible stress, or significant mortality. However, some cannibalism was noticed especially if the holding facilities contained insufficient vegetation cover. In spite of this, a survival rate of approximately 80-85% was noted for the gammarids, throughout the four-month period.

## Lobsters

**Berried Females.** Berried female lobsters were collected approximately 1.5 km west of the plant operations 21-28 May and additional lobsters were collected as required in late June. Each lobster was measured, weighed and tagged around one walking leg prior to being placed into the lobster nursery.

Eye indices of the fertilized eggs were calculated using a method developed by Perkins(2) to estimate the time to hatching. The lobsters were placed into individual nurseries with rock shelters and held at ambient water temperatures to permit egg development to proceed at a normal rate.

Lagoon water was supplied continuously at a rate of 4 L/min per nursery, and aerated to maintain 7-10 mg/L dissolved oxygen levels. Hatchery water temperature ranged from 9.5-20.0°C, $\bar{x}$:13.2°C over the four month period. The lobsters were fed one half rock crab weekly; the unconsumed portion was removed.

One of the four nursery females died after 10 days (cause unknown) and was replaced. The remaining berried females remained in excellent condition during their 1-8 week period of captivity, and were released at their point of capture once hatching was completed.

**Larvae.** Upon hatching, the free swimming and photopositive first stage larvae remained near the surface and floated via the notched section of the partition into the larval catch box which contained San Francisco Bay Brand brine shrimp for food. Initially-hatched larvae from each female were discarded (quantity too small). When a large number of larvae were present in the larval catching tray, they were removed, and placed in a dissecting tray. Batches of 2000 larvae were separated using a glass probe and placed into a Hughes* rearing tank (the optimum number for an 85% survival rate(3), keeping each female's brood segregated.

The larvae were initially fed a diet of brine shrimp 4 times a day according to a feeding schedule supplied by the St. Andrew's Biological Station. Increases in the recommended volumes of shrimp were implemented when increased cannibalism indicated that the larvae were not receiving sufficient food. The water flow was maintained at a rate of ~4-6 L/min (temperature range 12.1-21.0°C; $\bar{x}$: 15.5°C) and the dissolved oxygen levels ranged from 9.0-12.0 mg/L over the four month period. Generally at one week intervals, the larvae were transferred into an unused rearing tank to allow the original tank to be emptied, cleaned and rinsed with salt water to remove the accumulated solids.

By using eight Hughes* rearing tanks, it was possible to maintain the larvae hatched from each female in separate vessels, and thereby meeting the criterion that genetically similar organisms should be used in each test (4). Once the larvae began to change to stage 4, they were immediately transferred to another Hughes* rearing tank, preventing the more advanced stage larvae from preying upon the slower developing larvae. Care was taken to maintain genetically similar stage 4 larvae together.

The larvae reached the fourth stage in approximately four weeks at the laboratory water temperature ($\bar{x}$: 15.5°C) which was found to be similar to findings by others(4). Development to the fourth larval stage requires about two weeks at 20°C or three-four weeks at 15°C(5). The larvae remain in stage four for a further ten-twelve days prior to changing to stage five(5).

Generally, stage 3 larvae are preferred for bioassays because their natural mortality rate is lower than for the earlier stages; yet they are a sensitive planktonic stage(5). The stage 4 larvae however, are the first stage in which the resemblance to a mature lobster is evident, and are also suitable for bioassays. Four days or so after reaching stage 4, the larvae (about 11-14 mm long and approximately 28-36 days old) were used in the test work.

Acclimation to bioassay dilution water conditions prior to testing, was unnecessary, since the larvae were reared in this water at ambient temperatures. A survival rate of 11-12.5% to stage 4 was observed for the lobster larvae, as compared to the 85% theoretical survival indicated by Hughes(3) and 25-55% observed survival at the St. Andrews Biological Station. Survival can be affected by a number of factors (drop in water supply, accumulation of suspended solids, improper feeding, disease) and survival as low as 1% has been found in St. Andrews using a closed system(6).

## Bioassay Methodology (1,7)

**General Bioassay Methodology for Gammarids.** Ten adult gammarids were exposed to test conditions (static - no replacement) in glass jars containing a 3.5 L volume of lagoon water (control) or test solution. Temperature was maintained by water bath at 18 ± 3°C and no aeration was provided during the 96-h exposure period. The jars were covered with plastic petri dishes to reduce evaporation and prevent the escape of any organisms. Each concentration, including the control, was conducted in duplicate. Selection of the test concentration range and increments were based on range-finding tests.

Random series of 10 gammarids were counted out from the holding tank approximately 1/2-1 hour before the start of the bioassay. They were placed into clean 16-oz glass jars held in the water bath, each containing 200 mL of seawater (portion of the dilution water) prior to random distribution into the test solutions.

Initial 48-h and final physico-chemical parameters were recorded with the following instruments: pH - Cole Parmer Digi-Sense pH meter; salinity/conductivity - YSI Model 33; dissolved oxygen/temperature - YSI Model 51B.

Mortalities were recorded and the dead organisms were removed at 11 logarithmic intervals. The gammarids were considered dead when no movement could be elicited (even if removed with a wide bore pipette, placed in a clean glass beaker and gently prodded with a glass probe). If any movement was observed, they were placed back into the test vessel.

A final set of parameters were determined at the end of the 96 hours, or earlier if all the organisms had died. Control mortality over 10% was not noted for any of the bioassays conducted with gammarids which otherwise would have rendered the test results invalid(1).

The mortality data at each test concentration was plotted on probability paper. The best-fit line was statistically determined and the 96-h $LC_{50}$ values was interpreted.

**General Bioassay Methodology for Lobster Larvae.** Unaerated 96-h static bioassays without replacement were conducted by individually exposing larva to 800 mL of test solutions in 16-oz glass jars. Concentration ranges were based on range-finding tests.

Typically, 8500 mL of each test solution was prepared in a 9000-mL polypropylene bucket. The initial parameters were determined (as per the gammarid bioassays) prior to subdividing the test concentration into the ten 16-oz glass jars calibrated to 800 mL. Once all the concentrations had been prepared, one stage 4 larvae was introduced to each test vessel with a dip net. The vessel was then covered with a plastic petri dish to reduce evaporation and prevent the escape of the larvae. Groups originating from the same brood stock, to ensure genetically similar organisms, were used in bioassays for each concentration.

The larvae were not fed over the course of the 96-h test. The test vessels were maintained in a water bath (18 ± 3°C) for the duration of the bioassay.

The 48-h and final physico/chemical parameters were determined in 3 randomly-selected test vessels for each concentration tested.

Mortalities were recorded and the dead organisms removed at 11 logarithmic intervals. The lobster larvae were considered dead when no movement could be detected, even when gently prodded with a glass probe. The larvae were considered to be in stress if they were prostrate with only slight appendage movement. $LC_{50}$ values were determined as described previously (Section 2.3.1).

**Test Materials**

**Plant Effluent (Unsettled and Decant).** Effluent samples were collected on eleven different occasions from May to August, one to five days prior to the effluent bioassays. The samples were collected during or before one of the three phases of plant operations: during normal operations (between the weeks of 16 May and 12 June inclusive); prior to annual plant shutdown (week of 16 June): during the plant startup (week of 4 August 1980). The samples were placed in plastic-lined, sealed 20-L polypropylene pails and stored in the water bath until used. Initial physico-chemical parameters were recorded for each sample of effluent at the time of collection.

Typically, two 20-L pails of effluent were collected on ten of the eleven occasions, with the one exception being on June 19, just prior to the annual shutdown, where five 20-L pails of effluent were collected.

Three types of static effluent bioassays were conducted with gammarids and lobster larvae during the bioassay program: on fresh unsettled effluent, collected just prior to each test; on stored unsettle effluent to determine if the effluent toxicity changed with time; and on effluent decant (the liquid portion of fresh and stored settled effluent) to determine if the solid fraction of the effluent contributed to the toxicity of the whole effluent.

Subsamples of effluent for each bioassay were submitted to the BF Analytical Laboratory for determination of total and dissolved levels of fluoride, $P_2O_5$ and total solids, while the Brunswick Smelting Analytical Laboratory conducted heavy-metal analyses (samples preserved with 0.2% $NHO_3$ - Ultrex).

Prior to each bioassay, the effluent was agitated to resuspend any solids, except for the bioassays conducted with effluent decant. The appropriate portion of effluent was added to the test vessels and made up to volume (3.5 L and 8.5 L for gammarids and lobster larvae, respectively) with dilution water.

**Phosphoric Acid.** Phosphoric acid was selected as a toxicant because it is one of the major constituents of the BF plant effluent.

A series of dilutions of 62.17% phosphoric acid (Fisher Scientific reagent grade, certified ACS) with lagoon water provided the 5000 mg/L (as $P_2O_5$) stock solution.

Test solutions for both the gammarids and lobster larvae were made by adding, with a pipette, a predetermined volume of the stock solution, to an appropriate volume of dilution water.

**Hydrochloric Acid (re pH).** Exposure to various pH conditions, of gammarids and lobster larvae was selected as a test series since it was presumed that pH was a significant lethal characteristic of the effluent.

Department of Fisheries and Oceans recommended the use of hydrochloric acid in a flow-through system, since the chance of precipitation of the constituents of the dilution water would not be as great as with the use of phosphoric or sulphuric acids. The bioassay facilities at BF were not designed to conduct flow-through bioassays, therefore static bioassays were conducted with gammarids and lobster larvae to establish lethality curves.

The stock solution was 5% by volume 38% reagent grade hydrochloric acid (Fisher Scientific reagent ACS) in salt water. Test solutions for both gammarid and lobster larvae bioassays were made individually, by adding volume of stock acid, with a micro burette, into a constantly stirred test solution, until the desired pH was achieved.

**Reference Toxicant (Not Used in 1980).** In the 1979 preliminary bioassay program, a reference toxicant, dodecyl sodium sulphate (DDS) was utilized for the following reasons: to measure the health and sensitivity of the test organisms, to establish standard test conditions, to compare the relative toxicity of other substances, and to permit comparison with the work of other investigators(8,9).

Review of the 1979 results of the DSS bioassays and the difficulties encountered ($O_2$ stripping, foaming, unreliable test concentrations) with this chemical, suggested that it was not suitable as a reference toxicant. Further review of the literature(10,11) supported not using DSS as a reference toxicant.

Consultations with Dr. V. Zitko (Department of Fisheries)(12), Dr. J.B. Sprague (U of Guelph)(13) and Dr. P. Wells (U of Toronto)(14), indicated that there were conflicting opinions on the use of reference toxicants. While Drs. Zitko and Wells promoted the use of copper as a reference toxicant, Dr. Sprague suggested that its toxicity could be modified by natural chelating agents in the test water. His recommendation was to use the control tests as a guideline to measure the acceptability of the test results.

Since there was considerable controversy over what material to use as a reference toxicant, it was decided to use the measure of control mortality as the criterion for accepting or rejecting the bioassay results.

**Dilution (Lagoon) Water.** Lagoon water was used for all dilutions as well as for the maintenance of the organisms and for the water baths. Prior to each bioassay, samples were submitted to the BF Analytical Laboratory for total and dissolved fluoride and $P_2O_5$ analyses and to the Brunswick Smelting Analytical Laboratory for total and dissolved heavy-metal analyses.

## RESULTS AND DISCUSSION

The results of the gammarid and lobster larvae bioassays are summarized and discussed briefly in the following two sections. The effluent components are considered in the third section. The overall results are then assessed in the final section.

### Gammarid Bioassays

**Fresh Unsettled Effluent.** Seven 96-h bioassays were conducted with fresh unsettled effluent over the period 20 May to 16 June (Table 1).

TABLE 1 GAMMARID BIOASSAYS WITH FRESH UNSETTLED EFFLUENT

| Test No. | Effluent No. | Date Tested | Concentration % | Concentration No. of Conc.[1] | 96-h $LC_{50}$ % Unsettled Effluent | Corresponding pH |
|---|---|---|---|---|---|---|
| 80-1 | E-1 | 20/5 | 0-8 | 8 | 3.7 ± 0.3[2] | 6.5 |
| 80-2 | E-2 | 20/5 | 0-8 | 8 | 4.0 ± 0.3 | 6.5 |
| 80-3 | E-3 | 27/5 | 0-8 | 8 | 4.0 ± 0.3 | 6.4 |
| 80-4 | E-4 | 27/5 | 0-8 | 8 | 4.3 ± 0.3 | 6.4 |
| 80-5 | E-5 | 2/6 | 0-8 | 8 | 7.5 ± 0.5 | 6.25 |
| 80-8 | E-6 | 9/6 | 0-15 | 10 | 9.2 ± 0.6 | 6.15 |
| 80-10 | E-7 | 16/6 | 0-15 | 10 | 8.1 ± 0.5 | 6.1 |

[1] Number of concentrations per test.
[2] 95% confidence limits.

Key comments are:

- Effluent toxicity was consistent during one day (Test 80-1/2, 80-3/4) but varied for weekly samples.

- The overall toxicity ranged from 3.4-9.8% effluent. The first four tests had a mean of 4.0%, while the mean of the three June tests was 8.3%. The effluent analyses do not indicate a reason for this difference; possibly the lower salinity of the dilution water in May was a factor (17 ppt* vs 23 ppt). Review of the literature indicated that a decrease in salinity generally increased the toxicity of the tested compounds(15-19).

- The corresponding pH values varied inversely with concentration over the range 6.1-6.5 which were generally below the pH range of 6.5-8.5 (with not more than 0.2 units outside the normally-occurring range for salt water) recommended by the 1976 U.S. EPA Quality Criteria for water(20). The main basis for these criteria was that plankton and benthic invertebrates are probably more sensitive than fish to changes in pH and the mature forms and larvae of oyster are adversely affected at the extremes of the pH range 6.5-9.0.

**Stored Unsettled and Decant Effluent.** A total of seven bioassays were conducted with one batch of effluent collected on 19 June and stored over a period of a month. Four tests were conducted with unsettled and three with decant effluent (Table 2).

**Phosphoric Acid.** Four bioassays were conducted with phosphoric acid in the range of 0-175 mg/L as $P_2O_5$ (corresponding to pH 8.2-2.7). Results are shown in Table 3.

**Hydrochloric Acid.** Three bioassays were conducted with hydrochloric acid over the pH range of 3.5-8.3 (Table 4).

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* parts per thousand

TABLE 2 GAMMARID BIOASSAYS WITH STORED UNSETTLED AND DECANT EFFLUENT

| Test No. | Effluent No. | Date Tested | Concentration | | 96-h $LC_{50}$ | | Corresponding pH |
|---|---|---|---|---|---|---|---|
| | | | % | No. of Conc.[1] | % Unsettled Effluent | % Decant Effluent | |
| 80-12 | E-8-4 | 23/6 | 0-15 | 10 | 6.1 ± 0.4[1] | | 6.5 |
| 80-13 | E-8-4D | 23/6 | 0-15 | 10 | | 7.1 ± 0.4[1] | 6.4 |
| 80-14 | E-8-5 | 1/7 | 0-15 | 10 | 7.2 ± 0.4 | | 6.2 |
| 80-15 | E-8-5D | 1/7 | 0-15 | 10 | | 8.0 ± 0.4 | 6.0 |
| 80-16 | E-8-6 | 7/7 | 0-15 | 10 | 7.1 ± 0.3 | | 6.1 |
| 80-17 | E-8-6D | 7/7 | 0-15 | 10 | | 8.5 ± 0.4 | 5.6 |
| 80-21 | E-8-8 | 21/7 | 0-15 | 10 | 6.5 ± 0.3 | | 6.0 |

[1] 95% confidence limits.

Key comments are:

- The toxicity of the stored unsettled effluent did not vary significantly over the one month period, nor did the toxicity of decant samples.
- The toxicity of unsettled effluent was significantly higher than for concurrent decant (means of 6.8% and 8.1%, respectively). The corresponding pH varied inversely with concentration for both types of effluent.
- The results indicate that one month storage had no measurable effect on toxicity, while the solid fraction did contribute to toxicity.

TABLE 3 GAMMARID BIOASSAYS WITH PHOSPHORIC ACID

| Test No. | Date Tested | Concentration | | 96-h $LC_{50}$ mg/L as $P_2O_5$ | Corresponding pH |
|---|---|---|---|---|---|
| | | mg/L as $P_2O_5$ | No. of Conc. | | |
| 80-7 | 2/6 | 0-175 | 8 | 73 ± 3[1] | 5.0 |
| 80-9 | 9/6 | 0-100 | 12 | 81 ± 1 | 4.9 |
| 80-11 | 16/6 | 0-100 | 12 | 76 ± 2 | 5.1 |
| 80-19 | 15/7 | 0-100 | 12 | 76 ± 2 | 5.1 |

[1] 95% confidence limits.

Key comments are:

- The results of test 80-9 was significantly different than for the other three tests; numerical difference was small.
- The toxicity range was 73-81 mg/L as $P_2O_5$.
- The corresponding pH values were about 5 (see comment in Section 6.1.1 re pH criteria).

TABLE 4 GAMMARID BIOASSAYS WITH HYDROCHLORIC ACID

| Test No. | Date Tested | Concentration pH | Concentration No. of Conc. | 96-h $LC_{50}$ pH | Corresponding mg/L as HCL |
|---|---|---|---|---|---|
| 80-32 | 11/8 | 4.0-7.60 | 5 | 4.88 ± 0.18[1] | 48.4 |
| 80-33 | 18/8 | 3.5-8.25 | 9 | 5.05 ± 0.08 | 48.2 |
| 80-34 | 18/8 | 3.5-8.20 | 9 | 4.98 ± 0.08 | 48.3 |

[1] 95% confidence limits.

Key comments are:

- The results were not statistically different.
- The toxic range for pH was 4.9-5.1 (similar to that noted for phosphoric acid). See Section 6.1.1 re pH criteria.

**Summary of Gammarid Bioassay Observations**

a) Fresh unsettled effluent toxicity varied over a one month period from 3.4-9.8%, possibly due to composition or salinity changes.

b) Repeat testing of a stored unsettled effluent batch indicated no significant variation in toxicity.

c) Comparison of stored unsettled and decant effluents indicated a significantly higher toxicity for the unsettled effluent (6.8% and 8.1% mean values, respectively), attributable to the higher solids content of the former (about 3% vs 1%).

d) Tests with phosphoric and hydrochloric acid both indicated toxicity at about pH 5 in the absence of effluent. For comparison, pH values corresponding to effluent $LC_{50}$'s ranged from 5.6 to 6.5, and varied inversely with effluent concentration.

**Lobster Larvae Bioassays**

**Fresh Unsettled and Decant Effluent.** Two tests each were conducted with unsettled and decant effluent collected at 8 a.m. and 1 p.m. on 4 August (Table 5).

**Stored Unsettled and Decant Effluent.** Six bioassays were conducted, five with unsettled and one with decant effluent from a batch collected on 19 June and stored for a month (Table 6).

**Phosphoric and Hydrochloric Acid.** One bioassay was conducted with phosphoric acid in the range of 0-120 mg/L as $p_2O_5$ (corresponding pH 7.5-3.1) and one with hydrochloric acid over the pH range of 4-7.6 (Table 7).

TABLE 5 LOBSTER LARVAE BIOASSAYS WITH FRESH UNSETTLED AND DECANT EFFLUENT

| Test No. | Effluent No. | Date Tested | Concentration % | Concentration No. of Conc.[1] | 96-h $LC_{50}$ % Unsettled Effluent | % Decant Effluent | Corresponding pH |
|---|---|---|---|---|---|---|---|
| 80-26 | E-9D | 4/8 | 0-15 | 7 | | 12.1 ± 0.8[1] | 5.6 |
| 80-27 | E-9 | 4/8 | 0-15 | 7 | 10.7 ± 1.3 | | 5.9 |
| 80-28 | E-10D | 4/8 | 0-15 | 7 | | 15.8 ± 1.7 | 5.2 |
| 80-29 | E-10 | 4/8 | 0-15 | 7 | 7.9 ± 0.7 | | 6.5 |

[1] 95% confidence limits.

Key comments are:

- There was a statistical difference between the results for the morning and afternoon samples, and between the afternoon unsettled and decant samples. (E-10 and E-10D).
- These results indicate (but do not establish) that the unsettled effluent was more toxic than the decant (mean differences of 1.4 and 7.9% for the two sets of tests).
- The mean toxicity value for unsettled effluent was 9.3% (at pH 6.2 and for decant effluent was 14.5% (at pH 5.4).

## Summary of Lobster Larvae Bioassay Observations

a) Fresh unsettled effluent $LC_{50}$ values were 10.7 and 7.9%, both of which were lower than the corresponding decant values of 12.1 and 15.8%; the results indicated (but did not establish) that the unsettled effluent was the more toxic.

b) For stored effluent, the mean $LC_{50}$ was 8.6% for five tests, compared to 10.1% for the one decant test. No significant difference was demonstrated.

c) The corresponding pH range for all effluent tests was 5.2 to 6.5 with a mean of 5.9 pH.

d) Both the phosphoric and hydrochloric acid bioassays indicated a toxic pH of about 5 in the absence of effluent.

## Effluent Composition

**Analytical Trends.** The ranges and means for the analyzed components of the fresh unsettled, stored unsettled and stored decant effluents are shown in Table 8. It is apparent from the wide fluctuations in the levels of the analyzed components found in repeat tests of the stored material and the absence of trends with time that a combination of sample variations and analytical variations obscure any numerical assessment of the relative importance of the measured variables. It is evident however, that the total values were significantly higher than the dissolved values; the relative

TABLE 6 LOBSTER LARVAE BIOASSAYS WITH STORED UNSETTLED AND DECANT EFFLUENT

| Test No. | Effluent No. | Date Tested | Concentration | | 96-h $LC_{50}$ | | Corresponding pH |
|---|---|---|---|---|---|---|---|
| | | | % | No. of Conc.[1] | % Unsettled Effluent | % Decant Effluent | |
| 80-18 | E-8-7 | 15/7 | 0-15 | 10 | 7.7 ± 0.5[1] | | 6.0 |
| 80-20 | E-8-8 | 21/7 | 0-15 | 10 | 8.7 ± 0.5 | | 6.2 |
| 80-22 | E-8-9 | 25/7 | 0-15 | 10 | 8.0 ± 0.6 | | 6.4 |
| 80-23 | E-8-10D | 28/7 | 0-15 | 7 | | 10.1 ± 0.6[1] | 5.2[2] |
| 80-24 | E-8-10 | 28/7 | 0-15 | 7 | 9.1 ± 0.8 | | 6.0 |
| 80-25 | E-8-10 | 28/7 | 0-15 | 7 | 9.5 ± 0.7 | | 6.0 |

[1] 95% confidence limits.

[2] Unexpectedly low value, no apparent explanation.

Key comments are:

- There was a slight statistical difference between the first three and last two unsettled bioassay results; it is questionable whether this indicates a change with age of the effluent, as the first set of effluents were 26-36 days old and the last two sets were 39 days old.
- The mean toxicity of unsettled effluent was 8.6% (corresponding pH of 6.1).
- The result of the decant test was not significantly different than for the parallel unsettled effluent tests (no evidence of effect of solids on toxicity).
- Test 80-20 was conducted with the same effluent sub-sample as Test 80-21 with gammarids. Toxicity to lobster larvae appeared lower (8.7% versus 6.5% for gammarids).

TABLE 7 LOBSTER LARVAE BIOASSAYS WITH PHOSPHORIC AND HYDROCHLORIC ACID

| Test No. | Date Tested | Concentration Range | No. of Conc. | 96-h $LC_{50}$ | Corresponding pH or mg/L as HCl |
|---|---|---|---|---|---|
| 80-31 | 11/8 | 0-120 mg/l as $P_2O_5$ | 7 | 81.2 ± 3.4[1] mg/L as $P_2O_5$ | pH5 |
| 80-30 | 11/8 | pH 4-7.6 | 5 | 5.0 ± 0.3 pH units | 48.2 mg/L as HCl |

[1] 95% confidence limits.

Key comments are:

- The $LC_{50}$ for phosphoric acid was 81.2 ± 3.4 mg/L as $P_2O_5$, corresponding to pH 5.
- The LC50 for hydrochloric acid was 5.0 ± 0.3 pH units, which is consistent with the phosphoric acid results.

TABLE 8 RANGES AND MEANS OF ANALYZED COMPONENTS IN BELLEDUNE FERTILIZER EFFLUENT USED IN STATIC BIOASSAYS, MAY-AUGUST, 1980

| Type of Sample | Number of Analyses[1] | Pb g/L | | Zn g/L | | Cd g/L | | F mg/L | | $P_2O_5$ mg/L | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Range | Mean | Range | Mean | Range | Mean | Range | Mean | Range | Mean |
| Stored Unsettled, Unfiltered | 8 | 142-445 | 312 | 240-1220 | 540 | 27-82 | 58 | 102-640 | 210 | 153-540 | 280 |
| Stored Unsettled, Filtered | 8 | 60-194 | 98 | 110-570 | 250 | 12-40 | 33 | 80-322 | 132 | 2-176 | 72 |
| Stored Decant, Unfiltered | 4 | 114-182 | 136 | 100-540 | 320 | 12-61 | 42 | 100-630 | 250 | 135-276 | 202 |
| Stored Decant, Filtered | 4 | 56-108 | 91 | 80-320 | 240 | 12-44 | 34 | 93-535 | 215 | 16-177 | 120 |
| Fresh Unsettled, Unfiltered | 10 | 144-549 | 370 | 250-1070 | 600 | 46-390 | 160 | 28-440 | 152 | 170-1011 | 498 |
| Fresh Unsettled, Filtered | 10 | 58-188 | 128 | 130-920 | 430 | 29-350 | 120 | 76-400 | 150 | 5-146 | 72 |

[1] For all stored samples (main sample collected 19 June 1980), analyses were conducted on sub-samples at intervals over a 7-week period.

The fresh samples were individual samples, collected at various dates from 16 May to 4 August 1980.

availability of the elements associated with the solid fraction during the period of the bioassays is not known.

### Relative Contribution of All Effluent Factors

a) pH

In the absence of effluent, the 96-h $LC_{50}$ was about pH 5 and in the presence of effluent, the related pH was typically 6 with 10 and 1 micro-equivalents of $H^+$ per litre respectively. This indicates that, for the effluent toxicities, pH values is significant but not the only factor. (Other factors are equivalent to a 1-unit decrease in pH, or 9 micro-equivalent of $H^+/L$).

b) Suspended Solids

The bioassays with unsettled and decant effluents indicated relative toxic volumes of 6.8 and 8% for gammarids. This suggests that solids account for perhaps 15% of the other toxicity. These tests did not establish whether the toxicity was physical (e.g. blocking of the gills) or chemical.

c) Analyzed Components

As indicated in Section 3.3.1, there was no clear indication in these tests of relationship between analysis of five components and toxicity. A computer literature search regarding the toxicity of these components in the marine environment was unsuccessful. Hence, no conclusions can be drawn regarding their contribution to effluent toxicity.

d) Summation

The pH, suspended solids and composition of the effluent were all contributors to the effluent toxicity. The relative roles could not be quantified or estimated based on either this work or the literature.

## OVERALL CONCLUSIONS

Plant effluent was found to be toxic to gammarids in the concentration range of about 4-10%, and to lobster larvae in the range of 8-11%. Decant effluent was slightly less toxic than unsettled effluent (contained approximately 1% versus 2-3% solids). Storage of effluent over a five-week period had no apparent effect on its toxicity. The corresponding effluent $LC_{50}$ pH values were approximately 6, as compared to a pH of 5 in experiments with both phosphoric and hydrochloric acid (effect of pH).

Table 9 summarizes the 96-h $LC_{50}$ value found in the 1980 bioassay program. The comments below are derived from these results.

TABLE 9 SUMMARY OF 96-h $LC_{50}$ VALUES[1]

| Test Conditions | | 96-h $LC_{50}$ Values[2] | |
|---|---|---|---|
| Material Tested | Units | Gammarids | Stage 4 Lobster Larvae |
| 1. Unsettled Effluent | % | 3.4-9.8 (7)[3] | 7.9-10.7 (2)[4] |
| 2. Decant Effluent | % | - | 12.1-15.8 (2)[4] |
| 3. Stored Unsettled Effluent | % | 6.1-7.2 (4)[3] | 7.7-9.5 (5)[4] |
| 4. Stored Decant Effluent | % | 7.9-8.5 (3)[3] | 10.1 (1)[4] |
| 5. Phosphoric Acid as $P_2O_5$ | mg/L | 73.0-81.0 (4) | 81.0 (1) |
| Corresponding pH | pH | 4.9-5.1 | 5.0 |
| 6. Hydrochloric Acid | pH | 4.9-5.1 (3) | 5.0 (1) |

Notes:

[1] See text regarding details of the test conditions.
[2] Bracketed numbers indicate the number of bioassays conducted.
[3] Effluent of pH of $LC_{50}$ value ranged from 6.0 to 6.5.
[4] Effluent pH of $LC_{50}$ value ranged from 5.2 to 6.5.

- The above results indicate that lobster larvae were slightly less sensitive than gammarids, and that both organisms were affected by the effluent pH, suspended solids and elements. However, the relative roles and importance of these parameters could not be quantified or estimated on the basis of the present work, or the literature.

- The methodology proved to be suitable for conducting on-site marine toxicity bioassays. Gammarids are the preferred test organisms as the on-site bioassays have indicated that they are similar in sensitivity, simpler to raise, more consistent in responses and are more cost effective than lobster larvae.

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# EFFECTS OF SUBLETHAL HCN ON EXOGENOUS YOLK PRODUCTION IN RAINBOW TROUT (SALMO GAIRDNERI)

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CYR, D.G., P. AYSOLA, and S.M. RUBY. 1985. Effects of sublethal HCN on exogenous yolk production in rainbow trout (Salmo gairdneri). Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 373-374.

The liver of rainbow trout Salmo gairdneri is an active site of synthesis for secondary yolk production during ovarian development. This source of secondary yolk, which is a complex calcium-bound lipophosphoprotein, is termed vitellogenin. It is transported via the serum to developing oocytes.

The present study examines the effects of 0.02 mg/L HCN on secondary yolk production in the liver of immature rainbow trout following induction with estradiol under laboratory controlled conditions. Serum calcium and phosphoprotein phosphorus were utilized as indicators of vitellogenin production and release by the liver.

Rainbow trout weighing between 25 to 35 g were induced with 5 mg/kg of estradiol on days 0 and 3 of the experiment, and cyanide exposure commenced on day 4. Experiments were performed at 12 ± 1.0 C, and fish maintained at a 12-hour light-12-hour dark photoperiod. Fish were sacrified on days 1,4,7,9,12 and 17 and serum samples analyzed for calcium and phosphoprotein content.

Estradiol-induced ($E_2$-induced) cyanide exposed fish demonstrated higher levels of serum calcium and phosphoprotein phosphorus relative to the estradiol-induced group by day 12. Serum phosphoprotein phosphorus rose to 277.0 ± 59.0 ug/ml in $E_2$-induced cyanide exposed fish, while in the $E_2$-induced fish, levels reached 168.0 ± 81.2 ug/ml. Similarly, calcium rose to 26.0 ± 3.6 mg-% relative to 15.9 ± 3.1 in the $E_2$-induced controls. By day 17 however, serum phosphoprotein phosphorus levels and calcium had declined to 144.9 ± 26.0 ug/ml and 20.1 ± 3.2 mg-% respectively in $E_2$-induced cyanide exposed fish, while levels of 241.0 ± 107.9 ug/ml and 24.2 ± 7.5 mg-% for serum phosphoprotein phophorus and calcium were recorded in the group which received no cyanide but estradiol induction.

The implications of these findings relative to yolk production in rainbow trout are discussed with respect to previous studies in this area, and the feasibility of utilizing this induction model as a tool for studying mechanisms of yolk production under toxic stress will also be discussed.

CYR, D.G., P. AYSOLA, and S.M. RUBY. 1985. Effects of sublethal HCN on exogenous yolk production in rainbow trout (*Salmo gairdneri*). Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 374-375.

Le foie de la truite arc-en-ciel *Salmo gairdneri* est un lieu actif de synthèse pour la production de vitellus secondaire en cours de développement ovarien. Cette source de vitellus secondaire, qui est une lipophosphoprotéine complexe liée au calcium, s'appelle la vitellogénine. Elle est transportée au moyen du sérum vers les oocytes en développement.

Notre étude a pour objet d'examiner l'effet de 0,02 mg/L de HCN sur la production de vitellus secondaire dans le foie de truites arc-en-ciel non adultes, après une induction par estradiol, dans des conditions de laboratoire contrôlées. Le calcium sérique et le phosphore de phosphoprotéine ont été utilisés comme indicateurs de production de vitellogénine et de sa libération par le foie.

Des truites arc-en-ciel pesant entre 25 et 35 g ont été induites avec 5 mg/kg d'estradiol aux jours 0 et 3 de l'experience, et soumises à l'exposition au cyanure le jour 4. Les expériences ont été effectuées à 12 ± 1,0°C, et les poissons ont été maintenus à une photopériode de 12 heures d'éclairage et 12 heures d'obscurité. Les poissons ont été sacrifiées aux jours 1, 4, 7, 9, 12 et 17 et leurs échantillons de sérum ont été analysés afin d'en déterminer le contenu en calcium et en phosphoprotéine.

Les poissons induits à l'estradiol et exposés au cyanure ont montré de plus hauts niveaux de calcium sérique et de phosphore de phosphoprotéine que le groupe induit à l'estradiol, au jour 12. le phosphore de phosphoprotéine sérique a été porté au niveau de 225,0 ± 59,0 g/mL chez le poisson induit à l'estradiol et exposé au cyanure, tandis que chez les poissons simplement induits à l'estradiol, les niveaux n'ont atteint que 168,0 ± 80,2 µg/mL. De la même façon, le calcium est monté à 26,0 ± 3,6 mg %, comparativement à 15,9 ± 3,1 chez des poissons témoins induits à l'estradiol. Mais au jour 17, les niveaux de phosphore de phosphoprotéine et de calcium sérique sont revenus à 144,9 ± 26,0 µg/mL et à 20,1 ± 3,2 mg % respectivement chez les poissons induits à l'estradiol et exposés au cyanure, tandis que des niveaux de 241,0 ± 107,9 µg/mL et 24,2 ± 7,5 mg % pour le phosphore de phosphoprotéine sérique et de calcium sérique ont été enregistrés dans le groupe induit à l'estradiol, mais non exposé au cyanure.

Ces résultats relatifs à la production de vitellus chez la truite arc-en-ciel sont analysés en fonction des études précédentes réalisées dans ce domaine, et de la possibilité d'utiliser le modèle d'induction comme outil d'étude du mécanisme de production de vitellus sous l'influence d'un stress toxique.

# ASSESSMENT OF MUTAGENICITY USING HAPLOID AND DIPLOID AMPHIBIAN EMBRYOS

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HART, D.R. 1985. Assessment of mutagenicity using haploid and diploid amphibian embryos. Can. Tech. Rep. Fish Aquat. Sci. 1368: p. 375.

Adult male South African clawed frogs, Xenopus, laevis, were mutagenized by 3 day immersion in aqueous solutions of ethyl methanesulfonate (EMS) or ethyl nitrosourea (ENU), or by exposure to gamma radiation. They were then spawned repeatedly at 2 week intervals with untreated females, and embryonic survival of the progeny was used to assess genetic damage. Peak dominant lethality occurred 5 weeks after EMS mutagenesis at 400 mg/l and 1-7 weeks after gamma ray mutagenesis at 1500 R. Gamma treatment produced permanent sterility at 15 weeks. ENU treatment at 75 mg/l did not result in clear dominant lethal effects.

Androgenetic haploid embryos were produced by UV-inactivation of the egg pronucleus following fertilization by a mutagenized male. Such haploids carry no maternal genes to mask induced recessive mutations. Recessive lethal effects were indicated by reduced haploid survival 1-13 weeks after mutagenesis with EMS, ENU or gamma radiation.

HART, D.R. 1985. Assessment of mutagenicity using haploid and diploid amphibian embryos. Can. Tech. Rep. Fish Aquat. Sci. 1368: p. 375.

Des grenouilles adultes mâles d'Afrique du Sud, *Xenopus laevis*, ont été mutagénisées par immersion de 3 jours dans des solutions aqueuses d'éthyl méthanesulfonate (EMS) ou d'éthyl nitrosourée (ENU) ou par exposition aux rayons gamma. On les a ensuite fait frayer de façon répétée à des intervalles de 2 semaines avec des femelles non traitées, puis on a évalué les perturbations génétiques à partir des embryons survivants de leur progéniture. Le sommet principal de létalité est apparu 5 semaines après la mutagénèse par l'EMS, à 400 mg/L et 1 à 7 semaines après mutagénèse par les rayons gamma, à 1 500 R. Le traitement gamma a entraîné à 15 semaines une stérilité permanente. Le traitement à l'ENU à 75 mg/L n'a pas entraîné d'effets létaux nettement dominants.

Des embryons androgénétiques haploides ont été produits par inactivation aux ultraviolets du pronucléus des oeufs à la suite de la fécondation par un mâle mutagénisé. Les haploides ne portaient pas de gène maternel risquant de masquer des mutations récessives induites. Les effets létaux récessifs ont été indiqués par une survie réduite des haploides 1 à 13 semaines après mutagénèse au moyen de l'EMS, l'ENU ou des rayons gamma.

# ASSESSMENT OF SUBLETHAL EFFECTS OF ATRAZINE ON ZOOPLANKTON

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KAUSHIK, N.K., K.R. SOLOMON, G. STEPHENSON, and K. DAY. 1985. Assessment of sublethal effects of atrazine on zooplankton. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 377-379.

The use of large aquatic enclosures or limnocorrals is currently being investigated as a suitable method for assessing the impact of and recovery from a pesticide contamination of aquatic ecosystems. To supplement this field research, a laboratory study was initiated to investigate possible effects of sublethal concentrations of atrazine on zooplankton life history parameters.

In one set of experiments, Daphnia magna contained in cups were observed daily to determine the effects of low atrazine (LA) concentration of 0.2 mg/L and high atrazine (HA) concentration of 2.0 mg/L on fecundity and longevity. Such observations were made on individuals from 6 generations. The average number of young produced per female in D. magna individuals exposed to atrazine for 21 days (a standard chronic exposure experiment used to predict life cycle results) did not differ from the controls in generations 1, 2, and 3, however in generations 4, 5, and 6 the LA and HA treatments significantly reduced the number of young produced. The validity of the 21 d chronic exposure experiments as a predictive tool will be discussed. The D. magna individuals exposed to atrazine for their entire lifespan showed that only the HA treatment significantly reduced the average number of young produced per female, the mean value for controls being 160.5 as against 69.9 for HA treatment. There was no effect of atrazine on time to first brood (maturation time). Individuals exposed to atrazine lived longer (LA - 46 d, HA - 51 d) than the controls (41 d) in this cup experiment.

In another set of experiments approximately 20 D. magna individuals were placed in a 4 L aquarium containing water from treated and untreated corrals. Observations were made twice weekly to obtain results comparable to those from the cup experiment. The number of young produced per individual over the average lifespan in the HA treatment (75.2) was significantly lower than that in the controls (271.89). However, unlike the cup experiment the average lifespan of individuals exposed to atrazine was significantly reduced. The difference in these results will be discussed.

Analyses of field samples did not show a significant numerical difference at the species level of the cladoceran zooplankton between the controls and atrazine-treated corrals. Therefore, mean dry weight estimates of four dominant zooplankton species (Bosmina longirostris, Ceriodaphnia lacustris, Diaptomus oregonensis, and Mesocyclops edax) were used as indicators of growth and biomass. However dry weight estimates of individuals from treated and untreated corrals did not differ significantly.

The number of eggs per individual and the percentage of ovigerous females in the population were determined for two zooplankton species from preserved samples collected from the treated and untreated limnocorrals. No ovigerous female *Diaptomus oregonensis* were found in samples from the HA-treated limnocorrals. Atrazine did not affect the number of eggs per individual in *Ceriodaphnia lacustris*, however the percentage of ovigerous females in the population increased. This finding is important as it may explain why cladoceran zooplankton do not show a numerical decline when exposed to atrazine. Although the number of young produced is reduced, there may be more female individuals producing young.

KAUSHIK, N.K., K.R. SOLOMON, G. STEPHENSON, and K. DAY. 1985. Assessment of sublethal effects of atrazine on zooplankton. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 377-379.

L'utilisation de vastes étendues d'eau fermées, ou limnocorrals, est actuellement à l'étude en vue de déterminer s'il s'agit d'une méthode convenable pour l'évaluation des effets de la contamination par les pesticides sur les écosystèmes aquatiques, et du rétablissement subséquent. Afin de compléter les recherches effectuées sur le terrain, une étude en laboratoire a été entreprise pour rechercher les effets possibles des concentrations sublétales d'atrazine sur les paramètres du cycle biologique du zooplancton.

Dans une série d'expériences, des *Daphnia magna* contenus dans des cupules ont été observés chaque jour afin de déterminer les effets de faibles concentrations d'atrazine (AF), soit 0,2 mg/L, et de concentrations élevées d'atrazine (AE), soit 2,0 mg/L, sur la fécondité et la longévité des sujets d'expérience. Ces observations ont été effectuées sur des individus provenant de 6 générations. Le nombre moyen de jeunes produits par femelle chez les *D. magna* exposés à l'atrazine pendant 21 jours (expérience d'exposition chronique standard utilisée pour prévoir les résultats obtenus sur le cycle vital) n'a pas été différent de celui des témoins pendant les générations 1, 2 et 3, tandis que dans les générations 4, 5 et 6, les traitements à l'AF et à l'AE réduisaient de façon significative le nombre de jeunes produits. Nous analyserons par la suite la validité des expériences d'exposition chronique de 21 jours comme outil de prédiction. Des individus *D. magna* exposés à l'atrazine pendant toute leur durée de vie ont montré que seul le traitement à l'AE réduisait de façon significative le nombre moyen de jeunes produits par femelle, la valeur moyenne des témoins étant de 160,5, contre 69,9 pour le traitement à l'AE. Aucun effet de l'atrazine n'a été observé jusqu'au moment de la première génération (temps de maturation). Les individus exposés à l'atrazine ont vécu plus longtemps (AF, 46 jours; AE, 51 jours) que les témoins (41 jours) dans cette expérience sur cupules.

Dans un autre ensemble d'expériences, environ 20 individus *D. magna* ont été placés dans un aquarium de 4 L contenant de l'eau provenant de corrals traités et non traités. Des observations ont été faites deux fois par semaine, afin d'obtenir des résultats comparables à ceux de l'expérience sur cupules. Le nombre de jeunes produits par individu sur une durée moyenne de vie dans le cas du traitement à l'AE (75,2) a été significativement inférieur à celui observé chez les témoins (271,89). Par contre, à la différence de l'expérience des cupules, la durée de vie moyenne des individus exposés à l'atrazine a été réduite de façon significative. La différence entre ces résultats sera analysée.

L'analyse des échantillons sur le terrain n'a pas montré de différence numérique significative au niveau de l'espèce du zooplancton cladocère, entre les corrals témoins et les corrals traités à l'atrazine. Par conséquent, une estimation du poids sec de quatre espèces dominantes de zooplancton (Bosmina longirostris, Ceriodaphnia lacustris, Diaptomus oregonensis, et Mesocyclops edax) a été utilisée comme indicateur de croissance et de biomasse. Mais les estimations de poids sec des individus provenant de corrals traités et de corrals non traités n'ont pas montré de différence significative.

Le nombre d'oeufs par individu, et le pourcentage de femelles ovigères dans la population ont été déterminés pour deux espèces de zooplancton provenant d'échantillons préservés recueillis dans les limnocorrals traités et non traités. Aucune femelle ovigère de Diaptomus oregonensis n'a été trouvée dans les échantillons provenant de limnocorrals traités à l'AE. L'atrazine n'a pas modifiée le nombre d'oeufs par individu chez le Ceriodaphina lacustris, bien que le pourcentage de femelles ovigères ait augmenté dans la population. Ce dernier résultat est important, car il pourrait expliquer pourquoi le zooplancton cladocère ne montre pas de décroissance numérique lorsqu'il est exposé à l'atrazine. Bien que le nombre de jeunes produits soit réduit, il peut arriver que plus de femelles produisent des jeunes.

# THE DEVELOPMENT OF ACUTE, WHOLE, AND PARTIAL LIFE CYCLE COPEPOD ASSAYS FOR HAZARD ASSESSMENT STUDIES

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MARCY, M. and D.C. MILLER. 1985. The development of acute, whole, and partial life cycle copepod assays for hazard assessment studies. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 381-382.

Hazard assessment is one approach currently being explored by the U.S. Environmental Protection Agency to systematically gather information on the environmental problems associated with ocean disposal of waste materials. Waste characterization involves first tier tests, including 96h assays, to screen for the relative hazards of potentially toxic chemicals. More elaborate predictive assays such as partial chronic and whole life cycle studies reveal more subtle, long term effects. Copepods may be ideal organisms for such testing due to their availability, ecological importance, relatively short lifespan, and their sensitivity to many pollutants. Either laboratory cultured or field caught animals may be used, although a laboratory population is advantageous, providing year- around availability and the possibility for age or stage standardization.

Topics treated will include culture, establishment of laboratory populations as "surrogates" for oceanic copepods through comparative toxicology, and modeling to extrapolate chronic assay results to the population level. Selected results will be presented on whole life cycle responses of *Eurytemora affinis* to nine temperature-salinity combinations; LC50 estimates for copper and cadmium in *E. affinis* nauplii and copepodids will also be cited. Ongoing work with the coastal and oceanic copepods *Eurytemora herdmani* and *Pseudocalanus minutus*, for sewage sludge toxicity assessment, will also be discussed.

MARCY, M. and D.C. MILLER. 1985. The development of acute, whole, and partial life cycle copepod assays for hazard assessment studies. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 381-382.

Les évaluations de risques constituent une méthode qu'étudie actuellement l'Environmental Protection Agency des États-Unis, en vue de rassembler systématiquement des données sur des problèmes environnementaux associés au rejet de déchets en mer. La caractérisation de ces rejets comprend des essais de premier échelon, comme les essais sur 96 heures, afin de rechercher les risques relatifs que présentent des produits chimiques potentiellement toxiques. Des essais de prédiction plus perfectionnés, tels que des études partielles, chroniques et sur le cycle biologique entier font apparaître des effets à long terme plus subtils. Les copépodes peuvent être des organismes idéaux pour de tels essais, en raison de leur disponibilité, de leur importance écologique, et de leur

durée de vie relativement courte, ainsi que de leur sensibilité à de nombreux polluants. On a utilisé soit des animaux cultivés en laboratoire ou pris dans la nature, mais une population de laboratoire présente des avantages, car elle est disponible à longueur d'année et il est possible d'en normaliser l'âge ou le stade.

Parmi les sujets traités figurent la culture, l'établissement de populations de laboratoire comme "substituts" de copépodes océaniques pour des études de toxicologie comparative, et l'établissement de modèles, en vue d'extrapoler les résultats d'essais chroniques à l'échelle de populations. Les résultats choisis sont présentés sur les réactions pendant toute la durée du cycle biologique de Eurytemora affinis à neuf combinaisons de température et de salinité; sont également envisagés chez des nauplii et copépodites E. affinis les évaluations de la CL50 pour le cuivre et le cadmium. Nous analyserons également le travail en cours qui porte sur les copépodes côtiers et océaniques Eurytemora herdmani et Pseudocalanus minutus, en vue de l'évaluation de la toxicité des boues d'égout.

# SUBLETHAL AND ACUTE TOXICITY OF CYANIDE TO EXERCISED AND NON-EXERCISED RAINBOW TROUT (*SALMO GAIRDNERI*)

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MCGEACHY, S. and G. LEDUC. 1985. Sublethal and acute toxicity of cyanide to exercised and non-exercised rainbow trout (*Salmo gairdneri*). Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 383-384.

Lethal toxicity (96 hr LC50) of cyanide (HCN) to fingerling rainbow trout (*Salmo gairdneri*) varied seasonally and with exercise (forced to swim at 1 body length/sec). The trout were acclimated to 12°C test temperature for 3-4 weeks and kept under a 12 hour photoperiod. During summer experiments, there was no significant difference in LC50 between exercised and non-exercised trout (.058 and .056 mg/l HCN respectfully). However, in winter trials, the LC50 for exercised trout was higher (.052 mg/l compared to .042 mg/l). Median survival times were similar in the summer while those of the exercised fish exceeded that of the non-exercised fish by up to 100% during the winter assay.

Glycogen depletion and growth rates were compared between the exercised and the non-exercised trout subjected to sublethal (.005, .010, and .020 mg/l) levels of HCN. A 20 day sublethal growth study indicated that cyanide (HCN) had no affect on liver glycogen depletion. However, the glycogen levels in the exercised trout were 53% lower than the non-exercised fish. Increasing daily ration from 0.5% to 2.5% body wt/day caused a less severe glycogen reserve differential (17%) between exercised and non-exercised fish. Cyanide exposed exercised trout showed increased growth rates over that of the control. The non-exercised fish grew more slowly at .010 and .020 mg/l but growth was similar at .005 mg/l. The non-exercised trout grew faster (16.7%) than the exercised trout when no cyanide was present.

MCGEACHY, S. and G. LEDUC. 1985. Sublethal and acute toxicity of cyanide to exercised and non-exercised rainbow trout (*Salmo gairdneri*). Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 383-384.

La toxitié létale (CL50 - 96h) du cyanure (HCN) sur des truitelles arc-en-ciel (*Salmo gairdneri*) a varié de façon saisonnière et en fonction de l'exercice (les poissons étaient forcés de nager à la vitesse d'une longueur par seconde). Les truites ont été acclimatées à la température d'essai de 12 °C pendant 3 à 4 semaines et gardées sous photopériode de 12 heures. En cours des expériences pratiquées l'été, on n'a noté aucune différence significative des CI50 entre truites soumises ou non à l'exercice (0,058 et 0,056 mg/L, de HCN, respectivement). Par contre, dans les essais d'hiver, la CL50 pour les truites soumises à l'exercice a été plus forte (0,052 mg/L, contre 0,042 mg/L). Les temps de survie médians ont été similaires l'été, tandis que ceux des poissons soumis à l'exercice

ont dépassé ceux des poissons non soumis à l'exercice de plus de 100 %, pendant les essais d'hiver.

Perte de glycogène et taux de croissance ont été comparés entre les truites soumises ou non soumises à l'exercice et exposées à des taux sublétaux de HCN (0,005, 0,010 et 0,020 mg/L). Une étude de croissance sublétale de 20 jours a indiqué que le cyanure n'avait pas d'action sur la perte de glycogène hépatique. Par contre, les niveaux de glycogène étaient inférieurs de 53 %, chez les truites soumises à l'exercice, à ceux des truites qui ne l'étaient pas. Un accroissement de la ration journalière, passant de 0,5 % à 2,5 % du poids corporel par jour, a provoqué une baisse de la différence des réserves de glycogène entre poissons soumis à l'exercice et poissons non soumis à l'exercice. Les truites soumises à l'exercice et exposées au cyanure ont présenté des taux de croissance accrus par rapport à celles du lot témoin. Les poissons non soumis à l'exercice ont eu une croissance plus lente aux concentrations de 0,10 et 0,20 mg/L, mais la croissance était semblable à la concentration de 0,05 mg/L. Les poissons non soumis à l'exercice ont grandi plus rapidement (16,7 %) que les poissons soumis à l'exercice, en l'absence de cyanure.

# BIOCONCENTRATION FACTOR (BCF): AN EXAMINATION OF ITS APPLICATION FROM A CHEMICAL AND BIOLOGICAL PERSPECTIVE

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NIIMI, A.J. 1985. Bioconcentration factor (BCF): an examination of its application from a chemical and biological perspective. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 385-386.

The bioconcentration factor (BCF) is a ratio between the chemical concentration in an organism and the concentration of the medium it inhabits. Estimates of BCF have been derived from laboratory studies and some field measurements. Values for the more environmentally relevant contaminants can range from 16 (log 1.20) for pentachlorophenol to 194,000 (log 5.28) for Aroclor 1260.

BCF's have been correlated with the chemical properties of a substance, the most common being the octanol-water partition coefficient. Comparative analyses have suggested the relationship between log octanol-water and log BCF to be linear with a regression coefficient (b) of 0.7 to 1.0.

Recent studies have indicated that BCF can be used to accurately predict concentrations of certain contaminants in fish from the natural environment. Some physical, chemical and biological factors that can influence the application of BCF as a useful index for chemical hazards will be discussed.

NIIMI, A.J. 1985. Bioconcentration factor (BCF): an examination of its application from a chemical and biological perspective. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 385-386.

Le facteur de bioconcentration (FBC) est le rapport entre la concentration chimique d'un produit dans l'organisme et sa concentration dans le milieu où se trouve cet organisme. Les estimations du FBC se font à partir d'études en laboratoire et de quelques mesures sur le terrain. Ses valeurs pour les contaminants le plus souvent rencontrés dans l'environnement vont de 16 (log 1,20) pour le pentachlorophénol à 194 000 (log 5,28) pour l'Aroclor 1260.

Le FBC a été associé aux propriétés chimiques d'une substance, la plus souvent employée étant le coéfficient de partage octanol-eau. Des études comparatives ont semblé indiquer que le rapport entre log octanol-eau et le log FBC serait linéaire avec un coefficient de régression (b) de 0,7 à 1,0.

Des études récentes ont montré que le FBC pouvait être utilisé pour prédire avec précision les concentrations chez les poissons de certains contaminants provenant de

l'environnement naturel. Nous examinerons certains facteurs physiques, chimiques et biologiques qui peuvent influencer l'application du FBC comme indice utile d'évaluation des dangers des produits chimiques.

# TOXICITY RESPONSES IN A FOOTHILL STREAM TO EFFLUENT FROM A BLEACHED KRAFT PULP MILL

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REYNOLDSON, T.B., J. KOSTLER, R.S. ANDERSON, and T. RICHEY. 1985. Toxicity responses in a foothill stream to effluent from a bleached Kraft pulp mill. Can. Tech. Rep. Fish. Aquat. Sci. 1368: p. 387.

The effects of effluent from a bleached kraft pulp mill at Grande Prairie, Alberta, on the Wapiti River have been monitored since operation began in 1973. Chemical and biological monitoring showed no measurable impact on the stream communities until 1979. Low stream flows, and a changed operational procedure resulting in a much more colored effluent in 1980 produced a significant change in downstream water quality and there was evidence of either a toxic or avoidance response in the stream benthic community.

Stream and effluent characterization is ongoing in 1983, using algal bioassays, stream chemistry and benthic invertebrate community structure. The extent of instream toxicity and the responsible components are being quantified. The value of the algal assay toxicity test is discussed with regard to establishing instream water quality criteria, and the instream processing of the effluent is discussed.

REYNOLDSON, T.B., J. KOSTLER, R.S. ANDERSON, and T. RICHEY. 1985. Toxicity responses in a foothill stream to effluent from a bleached Kraft pulp mill. Can. Tech. Rep. Fish. Aquat. Sci. 1368: p. 387.

Les effets de l'effluent d'une fabrique de pâte à papier kraft blanchi située à Grande Prairie (Alberta) sur la rivière Wapiti ont été observés depuis le début des activités de la fabrique en 1973. Les contrôles chimiques et biologiques n'ont montré aucun effet mesurable sur les populations de la rivière jusqu'à 1979. Le faible débit de la rivière, ainsi qu'un changement de procédé de fabrication ont entraîné en 1980 la formation d'un effluent beaucoup plus coloré, qui a eu des effets marqués sur la qualité de l'eau en aval; de plus, on a noté une réaction à la toxicité ou un comportement d'évitement dans la communauté benthique de la rivière.

En 1983, la rivière et l'effluent sont analysés et soumis à des dosages biologiques sur les algues, à une étude chimique de l'eau de la rivière, ainsi que la structure des communautés d'invertébrées benthiques. L'ampleur de la toxicité du cours d'eau et les composants responsables sont quantifiées. La valeur du test de toxicité par essai sur des algues est analysée en vue d'établir des critères de qualité d'eau de la rivière et on étudie les possibilités de traitement de l'effluent rejeté dans la rivière.

# POSTERS

Michael Hutcheson, Chairman

# SELECTIVE INDUCTION OF MFO IN FLOUNDER (PSEUDOPLEURONECTES AMERICANUS) AT THE SITE OF THE BAIE VERTE, NEWFOUNDLAND, OIL SPILL

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BAULD, C., A. DEY, and J.F. PAYNE. 1985. Selective induction of MFO in flounder (Pseudopleuronectes americanus) at the site of the Baie Verte, Newfoundland, oil spill. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 389-390.

A biochemical index shown to be a useful indicator for petroleum contaminants is the mixed-function oxygenase system (MFO) found in fish. Fisheries and Oceans laboratories in Newfoundland and Nova Scotia are particularly interested in the feasibility of using such sensitive responses as MFO at offshore petroleum development sites, in order to aid in "red-flagging" or delineating zones of possible hydrocarbon impact. Field studies establishing the sensitivity of the enzyme system to oil contaminated water have been carried out in Newfoundland (Payne, Science 191 945, 1976), the Mediterranean (Kurelec et al., Mar. Biol. 44 211, 1977), the USA (Stegeman, J. Fish. Res. Board Can. 35 668, 1979), and the North Sea (Davies et al., Mar. Poll. Bull. 12, 412, 1981). More recently, Spies et. al., (Mar. Biol., 1982) have detected elevated MFO levels in fish taken near the natural oil seeps off California. In all of the field studies to date induction has been noted in liver tissues. We now have evidence for "selective" petroleum mediated enzyme induction in a flatfish species. During the winter of 1981, No. 2 fuel from a land-based spill entered the ice covered harbor at Baie Verte, Newfoundland. Flounder (Pseudopleuronectes americanus) collected in early June at the site of the spill had elevated kidney levels of MFO, whereas liver and gill levels were comparable with enzyme activities found in fish at control sites. Electron transport components including cytochrome $P_{450}$, $P_{450}$ reductase, and cytochrome $b_5$ reductase were also measured but the greatest differences were observed for the MFO component, benzo(a)pyrene hydroxylase. The observations on flounder at this particular oil spill site are of particular interest and expand the utility of MFO in biological monitoring studies.

BAULD, C., A. DEY, and J.F. PAYNE. 1985. Selective induction of MFO in flounder (Pseudopleuronectes americanus) at the site of the Baie Verte, Newfoundland, oil spill. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 389-390.

Le système oxygénase à fonction mixte (OFM) qu'on observe chez le poisson est un indice biochimique utile comme indicateur des contaminants pétroliers. Les laboratoires de Pêches et Océans à Terre-Neuve et en Nouvelle-Écosse se sont particulièrement intéressés à la possibilité d'utiliser les réactions de sensibilité que constitue l'OFM sur les lieux d'exploitation pétrolière en mer, afin d'aider à signaler et à délimiter les zones de répercussions possibles des hydrocarbures. Des études sur le terrain déterminant la vulnérabilité du système enzymatique à l'eau contaminée par le pétrole ont été menées à Terre-Neuve (Payne, Science 191 945, 1976), en Méditerranée (Kurelec et coll. Mar. Biol.

44 211, 1977), aux États-Unis (Stegeman, J. Fish. Res. Board Can. 35 668, 1979) et dans la mer du Nord (Davies et coll. Mar. Poll. Bull. 12, 412, 1981). Plus récemment, Spies et coll. (Mar. Biol., 1982) ont détecté des niveaux élevés d'OFM chez des poissons capturés près des fuites naturelles de pétrole au large de la Californie. Dans toutes les études sur le terrain réalisées jusqu'à présent, on a noté cette induction dans les tissus hépatiques. Nous avons maintenant la preuve d'une induction enzymatique sélective par le pétrole chez une espèce de poisson plat. Au cours de l'hiver 1981, du carburant no. 2 provenant d'une fuite localisée sur terre a pénétré dans le port recouvert de glace de la baie Verte (Terre-Neuve). La plie rouge (Pseudopleuronectes americanus) recueillie au début juin sur les lieux de la fuite montrait des niveaux élevés d'OFM dans le rein, tandis que les niveaux observés dans le foie et les branchies étaient comparables aux activités enzymatiques trouvées sur les poissons des lieux témoins. Des éléments de transport d'électrons comprenant cytochrome $P_{450}$, $P_{450}$ réductase et cytochrome $b_5$ réductase ont également été mesurés, mais les plus grandes différences ont été observées avec le composant d'OFM, benzo(a)pyrène hydroxylase. Les observations ainsi faites sur la plie rouge à cet endroit particulier de déversement de pétrole sont particulièrement intéressants et mettant en relief l'importance de l'OFM pour les études de contrôle biologique.

# DETERMINATION OF CHLORINATED PHENOLS IN WATER AND FISH TISSUE

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BARATH, A., D. SMITH, C. MALLARD, D. ORR, and G. OZBURN. 1985. Determination of chlorinated phenols in water and fish tissue. Can. Tech. Rep. Rish. Aquat. Sci. 1368: p. 391.

A reliable method for the quantitative determination of chlorinated phenols in large numbers of routine water and fish tissue samples is presented. The procedure has been used extensively in our laboratory for 2, 4, 6 - trichlorophenol, 2, 3, 5, 6 - tetrachlorophenol and pentachlrophenol. High extraction efficiency has been demonstrated with good linearity of response and a minimum of intermediate steps.

The chlorinated phenols are first isolated by extracting the acidified samples with hexane and then converted to the corresponding chlorinated phenol acetates by derivatization with a mixture of acetic anhydride and pyridine. The resulting hexane extracts containing the chlorinated phenol acetates are washed with distilled water to remove impurities and analyzed directly by electron capture gas-liquid chromatography using an internal standard method of analysis.

BARATH, A., D. SMITH, C. MALLARD, D. ORR, and G. OZBURN. 1985. Determination of chlorinated phenols in water and fish tissue. Can. Tech. Rep. Rish. Aquat. Sci. 1368: p. 391.

Nous présentons une méthode fiable de détermination quantitative des phénols chlorés qui se trouvent très fréquemment dans les échantillons courants d'eau de tissu de poisson. Cette méthode a été utilisée de façon extensive dans notre laboratoire pour le 2, 4, 6 - trichlorophénol et le 2, 3, 5, 6 - tétrachlorophénol ainsi que le pentachlorophénol. Nous avons obtenu une très bonne efficacité d'extraction, avec une bonne linéarité de réponse et un minimum d'étapes intermédiaires.

On isole d'abord les phénols chlorés par extraction des échantillons acides au moyen d'hexane et on les transforme en acétates de phénol chloré au moyen d'un mélange d'anhydride acétique et de pyridine. Les produits d'extraction par l'hexane contenant des acétates de chlorophénol sont alors lavés avec de l'eau distillée de façon à en retirer les impuretés, et sont analysés directement par chromatographie gaz-liquide et capture d'électrons au moyen d'une méthode interne standard d'analyse.

# RESEARCH ON THE DEVELOPMENT OF A STANDARDIZED ECOTOXICOLOGICAL TESTS ON MARINE NEMATODES.
# II. DEVELOPMENTAL INHIBITION AND MORTALITY AS CRITERIA FOR A TEST WITH MONHYSTERA MICROPHTHALMA AND DIPLOILAIMELLOIDES BRUCIEI

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BOGAERT, T., M. SAMOILOFF, and G. PERSOONE. 1985. Research on the development of a standardized ecotoxicological test on marine nematodes. II. Developmental inhibition and mortality as criteria for a test with Monhystera microphthalma and Diploilaimelloides bruciei. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 393-394.

Considering the scarcity of ecotoxicological test methods for the marine meiobenthos, research was initiated to develop a simple, inexpensive and standardized toxicity test with marine nematodes.

Two species were selected on the basis of their representativity, short generation time and ease of controlled culturing: Diploilaimelloides bruciei (two strains) and Monhystera microphthalma.

A standard culture medium in artificial seawater was developed for the two species; a technique has been worked out for long term storage of D. bruciei at very low temperatures (freezer).

The toxicity criteria selected are analogous to those used by Samoiloff for the nematode test with Panagrellus redivivus, namely: inhibition of the postembryonic development (as measured by the degree of completion of the last three molts) and mortality.

The experimental test procedure has been derived from that developed for Panagrellus redivivus. It consists of placing 100 juvenile-1 nematodes from a standard culture per groups of 10 in series of auto-analyzer cups containing 0.5 ml of culture medium with the toxicant, and incubating the latter at 30°C. When 50% of the nematodes in the control have reached the adult stage, the surviving nematodes are transferred to a microscope slide, heat-killed and stained in lactophenol-cotton blue.

Pictures of the nematodes on the slides are taken with a microfiche reader provided with a photocopier, at a magnification of approximately 100 x. The length of the nematodes is measured with a digitizer and automatically converted in a computer program to the corresponding developmental stage of the nematodes, i.e. juvenile 2, 3, 4 or adult. Simultaneously the percentage mortality and the degree of developmental inhibition are determined.

The protocol has been tested out on a series of chemicals and the results will be reported. The possibility of using the specific inhibition of the juvenile-4 to adult molt as an indication of mutagenicity is presently examined.

BOGAERT, T., M. SAMOILOFF, and G. PERSOONE. 1985. Research on the development of a standardized ecotoxicological test on marine nematodes. II. Developmental inhibition and mortality as criteria for a test with *Monhystera microphthalma* and *Diploilaimelloides bruciei*. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 393-394.

Étant donnée la rareté des méthodes d'essai écotoxocologique s'appliquant au meiobenthos marin, nous avons entrepris une recherche visant à mettre au point un test de toxicité simple, peu coûteux et normalisé pour les nématodes marins.

Deux espèces ont été choisies en fonction de leur représentativité, de leur court temps de reproduction et de la facilité de contrôler leur culture: *Diploilaimelloides brucieri* (deux souches) et *Mohystera microphthalma*.

Un milieu de culture standard dans de l'eau de mer artificielle a été mis au point pour deux espèces, ainsi qu'une technique pour la conservation à long terme de *D. bruciei* à très basse température (congélateur).

Les critères de toxicité retenus sont analogues à ceux qui ont été employés par Samoiloff pour le test sur les nématodes avec *Panagrellus redivivus*: inhibition du développement post-embryonaire, mesurée par le degré d'achèvement des trois dernières mues et la mortalité.

La méthode du test expérimental dérive de celle qui a été mise au point pour *Panagrellus redivivus*. Elle consiste à placer 100 nématodes jeunes-1 provenant d'une culture standard par groupes de 10 dans des séries de cupules d'auto-analyse contenant 0,5 mL de milieu de culture avec le produit toxique et à incuber ce dernier à 30 °C. Lorsque 50 % des nématodes témoins ont atteint le stade adulte, les nématodes survivants sont transférés sur une lame microscopique, tués par la chaleur et colorés au bleu pour coton lactophénolé.

Des images des nématodes sur les lames sont prises au moyen d'un lecteur de microfiches muni d'un photocopieur, à un taux d'agrandissement d'environ 100 fois. La longueur des nématodes est mesurée avec un numériseur, et automatiquement convertie par ordinateur en stades de développement correspondants des nématodes, c'est-à-dire jeune 2, 3, 4 ou adulte. Simultanément, on détermine le pourcentage de mortalité, ainsi que le degré d'inhibition du développement.

Ce protocole a été mis à l'essai sur une série de produits chimiques, et nous en donnerons les résultats. On étudie présentement la possibilité d'utiliser l'inhibition spécifique du passage de la forme jeune-4 à la forme adulte comme indication de mutagénicité.

# DEVELOPMENT OF A TOXICITY TEST FOR THE DETERMINATION OF MUTAGENIC ACTIVITY IN THE MARINE ENVIRONMENT: TERATOGENESIS IN A MARINE POLYCHAETE OPHRYOTROCHA LABRONICA

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BOGAERT, T., M. SAMOILOFF, and R. PULAK. 1985. Development of a toxicity test for the determination of mutagenic activity in the marine environment: teratogenesis in a marine polychaete Ophryotrocha labronica. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 395-398.

The small polychaete Ophryotrocha labronica is a well established test organism for acute and chronic toxicity tests (see review by Akesson, 1983). This species can be easily cultured in the laboratory, has a short life cycle (19 days at 25 degrees C), reproduces throughout the year and has a cosmopolitan distribution (Akesson 1973, 1978, 1980). We have initiated experiments to determine if O. labronica can be used to detect tissue-level effects of mutagenic compounds.

In initial experiments, it was found that young adults subjected to a 6 hour exposure to 0.02 M of ethyl methanesulfonate (EMS) develop tumors within 2 weeks. The tumors are produced at a frequency of at least 2% and occur in the head and pharynx region as well as in the post-pharyngeal segments. The tumors can be laterally, ventrally as well as dorsally located and their size can exceed the width or height of the animals, which makes them easily detectable.

As well, some of the segments formed after the exposure to EMS developed incompletely formed parapodia.

To further test the O. labronica assay, 30 young adults of the Naples I strain were grown for 10 days in 14 coded samples (kindly provided by Mr. Birkholz, Environmental Protection Service Alberta) diluted 50:1 in artificial seawater and fed with spinach. One of these samples contained material collected downstream of an industrial site, while the others contained various known and suspected mutagens. It was found that:

- The environmental sample from an industrial site produced 100% mortality within minutes.
- In a sample containing a final concentration of 2 ppm 2-nitrofluorene in 2% dimethylsulfoxide 100% of the animals developed an abnormal tumor-like pygidium while the newly added segments were small with in some cases incompletely formed parapods. This abnormal development was observed after day 5.

- The other samples were not toxic at this concentration and are presently being run at a higher concentration.

When any number of setigerous segments of O. labronica are amputated, the anterior piece of the animal, containing the head and pharynx, grows a complete new pygidium within 5-7 days, even when they are not fed. To determine if the regeneration of the anterior segments of O. labronica could serve as a model for the effects of contaminants on development, the anterior part containing at least the pharyngeal segments + 1 setigerous segment of 15 animals was placed for 6 days in autoanalyzer cups with 0.1, 0.01, 0.001, 0.0001 and 0.00001 M ethyl methanesulfonate (EMS) dissolved in artificial seawater. It was found that:

- Exposure to 0.01 and 0.1 M EMS caused 100% mortality.
- In 0.001 M 46% of the animals regenerated a complete pygidium 12% of the animals died and 42% of the animals failed to regenerate the pygidium or regenerated an abnormal pygidial structure.
- All of the animals regenerated a complete pygidium in the control, 0.0001 and 0.00001 M EMS.

These results indicate that the adult tissues of O. labronica have extremely sensitive responses to mutagens producing tumors, and abnormal development and regeneration. This sensitivity can be utilized for a simple, rapid, inexpensive bioassay for such effects in samples from marine environments.

BOGAERT, T., M. SAMOILOFF, and R. PULAK. 1985. Development of a toxicity test for the determination of mutagenic activity in the marine environment: teratogenesis in a marine polychaete Ophryotrocha labronica. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 395-398.

Le petit polychète Ophryotrocha labronica est un organisme de test bien connu pour des évaluations de toxicité aigue et chronique (voir revue par Akesson, 1983). Cette espèce peut facilement être mise en culture au laboratoire, elle a un cycle biologique court (19 jours à 25 °C), se reproduit tout au long de l'année et a une distribution cosmopolite (Akesson 1973, 1978, 1980). Nous avons mis en route des expériences visant à déterminer si O. labronica pouvait être utilisé pour déceler les effets de produits mutagéniques au niveau des tissus.

Dans une première expérience, on a noté que des jeunes adultes soumis à une exposition de 6 heures à 0,02 M d'éthyl métasulfonate (EMS) développent des tumeurs en 2 semaines. Ces tumeurs sont produites à une fréquence d'au moins 2 %, et sont localisées dans la région de la tête et du pharynx, ainsi que dans les segments post-pharyngiens. Les tumeurs peuvent se repérer sur les côtés, sur la face ventrale ou sur la face dorsale. Leur taille peut dépasser la largeur ou la hauteur des animaux, ce qui les rend facilement reconnaissables.

Par ailleurs, certains des segments formés après exposition à l'EMS ont développé des parapodes incomplètement formés.

Pour poursuivre nos essais sur O. labronica, 30 jeunes adultes de la souche Naples 1 ont été élevés pendant 10 jours dans 14 échantillons codés (obtenus grâce à la courtoisie de M. Birkholz, Service de protection de l'environnement de l'Alberta) dilués au 50° dans de l'eau de mer artificielle et nourris d'épinards. Un de ces éthantillons contenait des matières recueillies en aval d'un lieu industriel, tandis que les autres contenaient des produits mutagènes divers reconnus et suspectés. Voici ce qui a été observé:

- l'échantillon environnemental provenant d'un lieu indutriel a entraîné une mortalité à 100 % en l'espace de quelques minutes;
- dans un échantillon contenant une concentration finale de 2 ppm de 2-nitrofluorène dans du diméthylsulfoxide à 2 %, 100 % des animaux ont développé un pygidium anormal d'apparence tumorale, tandis que les segments nouvellement ajoutés étaient de taille réduite, avec dans certains cas des parapodes incomplètement formés. Ce développement anormal a été observé après le jour 5;
- les autres échantillons n'étaient pas toxiques à cette concentration, et ils sont actuellement à l'essai à des concentrations plus élevées.

Lorsqu'un certain nombre de segments porteurs de soie de O. labronica sont amputés, la partie antérieure de l'animal contenant la tête et le pharynx développe un pygidium nouveau complet dans l'espace de 5 à 7 jours, même en l'absence de nourriture. Afin de déterminer si la régénération des segments antérieurs de O. labronica pourrait servir de modèle pour les effets des contaminants sur le développement, la partie antérieure contenant au moins les segments pharyngiens plus un segment sétifère de 15 animaux a été placée pendant 6 jours dans un tube à auto-analyse, avec 0,1, 0,01, 0,001, 0,0001 et 0,00001 M éthyl méthanesulfonate (EMS) dissous dans l'eau de mer artificielle. Voici les résultats obtenus:

- l'exposition à 0,01 et à 0,1 M EMS a entraîné une mortalité à 100 %. À la concentration de 0,001 M, 46 % des animaux ont régénéré un pygidium complet, 12 % sont morts, et 42 % n'ont pu régénérer le pygidium ou en ont régénéré un de structure anormale;
- dans l'échantillon-témoin ainsi qu'aux concentrations 0,0001 et 0,00001 M d'EMS, tous les animaux ont régénéré un pygidium complet.

Ces résultats indiquent que les tissus adultes de O. labronica ont une réaction extrêmement sensible aux mutagènes produisant des tumeurs et présentement un développement et une régénération anormaux. Cette sensibilité peut être utilisée sous forme d'un dosage biologique simple, rapide et non coûteux permettant de déterminer ces effets sur des échantillons provenant de milieux marins.

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# A COMPARISON OF UPTAKE AND EXCRETION OF ORGANOCHLORINE PESTICIDES BY NEREIS VIRENS UNDER NORMOXIC AND HYPOXIC CONDITIONS

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BURRIDGE, L.E., K. HAYA, and A. MCINTYRE. 1985. A comparison of uptake and excretion of organochlorine pesticides by *Nereis virens* under normoxic and hypoxic conditions. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 399-400.

The Polychaete worm *Nereis virens* is tolerant of organochlorine pesticides (McLeese 1981). It is also known that some marine invertebrates, including worms, can survive extended periods of anoxia by switching to anaerobic metabolic pathways.

The bioaccumulation of organochlorines by *Nereis* under normoxic and hypoxic water conditions was investigated to define the role of anaerobic metabolism in the mechanism or organochlorine pesticide toxicity.

In a set of preliminary experiments, worms were exposed to 14C-DDT (4.3 and 3.3 ppb) and 14C dieldrin (1.92 and 2.2 ppb) in normoxic and hypoxic sea water and sampled periodically over a 96-hr period. The remaining worms were transferred to unspiked normoxic and hypoxic sea water for 96 hr. Concentration of 14 C in whole body homogenates were determined by liquid scintillation techniques.

The uptake of 14C-DDT by worms exposed in normoxic sea water was greater than in those exposed under hypoxic conditions. The rate of uptake of 14C-dieldrin, however, was greater from hypoxic sea water.

A seawater experiment was carried out to clarify this anomally. Worms were exposed to DDT (4.6 and 3.5 ppb), dieldrin (9.0 and 7.1 ppb), and a third organochlorine, endosulfan (64.3 and 55.4 ppb). Dissolved oxygen was measured throughout the experiment; normoxic sea water having a concentration of 9.74 mg/L and hypoxic sea water as follows: DDT, .88 mg/L; dieldrin, 1.1 mg/L; endosulfan, 1.4 mg/L. Sampling of worms was carried out as before except that sampling on the excretion phase was extended to cover a 336-hr period. The organochlorine pesticides in whole worm extracts are being determined by GC techniques. The results will be discussed in relation to a one-compartment uptake-excretion model.

BURRIDGE, L.E., K. HAYA, and A. MCINTYRE. 1985. A comparison of uptake and excretion of organochlorine pesticides by *Nereis virens* under normoxic and hypoxic conditions. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 399-400.

Le ver polychète *Nereis virens* a une bonne tolérance face aux pesticides organochlorés (McLeese 1981). On sait également que certains invertébrés marins, y compris les

vers, peuvent survivre à des périodes d'anoxie prolongées en recourant à des mécanismes métaboliques anaérobies.

La bioaccumulation des organochlorés par Nereis dans des conditions aquatiques normoxiques et hypoxiques a été étudiée afin de définir le rôle du métabolisme anaérobie dans le mécanisme de la toxicité des pesticides organochlorés.

Dans une série d'expériences préliminaires, des vers ont été exposés au DDT 14C (4,3 et 3,3 ppb) et à la dieldrine 14C (1,92 et 2,2 ppb) dans de l'eau de mer normoxique et hypoxique, et échantillonnés périodiquement sur une période de 96 heures. Les vers restants ont été transférés dans de l'eau de mer non mélangée normoxique et hypoxique pendant la même durée de 96 heures. Enfin, on a déterminé la concentration de 14C dans des homogénats de corps entiers, au moyen des techniques de scintillation liquide.

La fixation du DDT 14C par les vers exposés à de l'eau de mer normoxique a été plus importante que chez ceux qui étaient exposés à des conditions hypoxiques. Le taux de fixation de la dieldrine 14C, cependant, a été plus important dans l'eau de mer hypoxique.

Une deuxième expérience a eu lieu, en vue d'éclaircir cette anomalie. Des vers ont été exposés à du DDT (4,6 et 3,5 ppb), à de la dieldrine (9,0 et 7,1 ppb) et à un troisième organochloré, l'endosulfan (64,3 et 55,4 ppb). L'oxygène dissous a été mesuré tout au long de l'expérience : l'eau de mer normoxique ayant une concentration de 9,74 mg/L, et l'eau hypoxique : DDT, 0,88 mg/L; dieldrine, 1,1 mg/L; endosulfane, 1,4 mg/L. L'échantillonnage des vers a été effectué comme précédemment, sauf que l'échantillonnage à la phase d'excrétion a été prolongé sur une période de 336 heures. Les pesticides organochlorés dans les extraits de vers entiers ont été analysés au moyen de techniques de chromatographie en phase gazeuse. Les résultats seront examinés en fonction d'un modèle de fixation-excrétion à un compartiment.

# MODELS FOR THE JOINT EFFECTS OF TOXICANTS IN ACUTE LETHAL BIOASSAYS

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DE MARCH, B.G.E. 1985. Models for the joint effects of toxicants in acute lethal bioassays. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 401-403.

There are few defensible mathematical methods for predicting the effects of mixtures of toxicants. The cost, size, and mathematical complexity of multivariate experiments with mixtures has prevented scientists from developing a consistent conceptual framework for interaction studies. As a result, descriptions and legal standards exist for individual toxicants but seldom for combinations. The most common predictive multivariate model in use, the "tolerance addition" or "toxic units" model has had limited acceptance. Experiments testing this model have classified certain toxicant combinations as more-than- or less-than-additive, but the model provides no framework for quantifying the degree of interaction. Nevertheless, in view of the technical difficulties, it is likely that toxicologists will have to continue using presumptive estimation methods, that is methods based on untested assumptions, such as the tolerance addition model, to predict the joint effects of toxicants.

The models described in the posters represented an approach to relating biological response surfaces to tolerance-based estimation methods. The models are defined as "tolerance-based" since they are expressed in terms of tolerances and other critical concentrations of toxicants. Also, the models are derived from plausible response surfaces, thus they can be confirmed with factorial experiments or can be utilized by making assumptions about the shapes of the multivariate response surfaces. The use of some of these models requires knowledge of other critical concentrations such as the highest concentration not eliciting a response, the relative change in tolerance concentrations due to only the presence (not concentrations) of other toxicants, and/or the "co-tolerance" of two or more toxicants.

A constant, temporarily defined as the "co-tolerance", which can be derived from response surface constants, was proposed as an index describing the interactive component of the tolerances of two or more toxicants. This constant is as consistent, and as amenable to tabulation, as an LC50 value is.

The models are consistent with joint action terms and models described by previous authors. The terms simple and complex joint action, dependent and independent action, interaction, synergism, antagonism, tolerance addition, and response addition are applicable and form part of a general model. The "tolerance addition" model was the simplest in this general class of models, and the "response addition" model was also relatively simple.

The applicability of these models is being tested in balanced multivariate experiments with common lethal divalent and monovalent cations, usually metals, near 96-hour LC50 concentrations, using Gammarus lacustris (fresh water shrimp) as the test organism.

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Il existe peu de méthodes mathématiques acceptables permettant de prévoir l'effet de produits toxiques mélangés. Le coût, l'envergure et la complexité mathématique des expériences à multi-variables de ces mélanges ont empêché les scientifiques de mettre au point un cadre conceptuel cohérent pour l'étude des interactions. Il en résulte qu'il existe des descriptions et des normes légales pour l'étude de produits toxiques individuels, mais rarement pour les mélanges de ces produits. Le modèle le plus courant de prédiction à multivariables utilisé, le modèle d'addition de tolérance ou d'unités toxiques, n'a été accepté que sur une échelle limitée. Les expériences d'évaluation de ce modèle ont classé certains mélanges de produits toxiques comme "plus que la somme" ou "moins que la somme", mais le modèle ne donne pas de cadre permettant de quantifier le degré d'interaction. Cependant, en raison des difficultés techniques, il est probable que les toxicologues devront continuer d'utiliser des méthodes d'estimation présomptive, qui consistent à se baser sur des hypothèses non vérifiées, comme le modèle d'addition de tolérance, pour prédire les effets conjoints des toxiques.

Les modèles décrits sur les affiches représentent une méthode visant à relier les surfaces de réaction biologique aux méthodes d'estimation fondées sur la tolérance. Les modèles sont définis comme fondés sur la tolérance, du fait qu'ils sont exprimés en termes de tolérance et autres concentrations critiques des toxiques. D'autre part, les modèles dérivent de surfaces de réaction plausibles, et peuvent donc être confirmés par des expériences factorielles, ou être utilisés en faisant des hypothèses sur les configurations des surfaces de réaction multivariées. L'utilisation de certains de ces modèles demande la connaissance d'autres concentrations critiques, comme la plus forte concentration n'évoquant pas de réponse, la modification relative des concentrations de tolérance due seulement à la présence (non aux concentrations d'autres produits toxiques) ainsi que la cotolérance de deux produits toxiques ou plus.

Une constante, définie provisoirement par le terme de "cotolérance", qui peut être dérivée des constantes de surface de réaction, a été proposée comme indice du composant interactif des tolérances de deux produits toxiques ou plus. Cette constante est aussi cohérente et aussi adaptable à la tabulation que la valeur CL50.

Les modèles sont cohérents avec les conditions et les modèles d'action mixte décrits précédemment par d'autres auteurs. Les conditions d'action mixte simple et complexe, d'action dépendante et indépendante, d'interaction, de synergie, d'antagonisme, de somme de tolérance et d'addition de réaction sont applicables et forment une part du modèle général. Le modèle "de somme de tolérance" a été le plus simple dans cette catégorie générale de modèles, et le modèle d'addition de réaction" a également été relativement simple.

L'application de ces modèles est actuellement à l'essai dans des études à multivariables équilibrées portant sur des cations létaux usuels bivalents et monovalents, généralement des métaux, à des concentrations proches des CL50 - 96 h, sur Gammarus lacustris (crevette d'eau douce) comme organisme test.

# BIOTRANSFORMATION ENZYME ACTIVITIES IN RAINBOW TROUT TREATED WITH CADMIUM

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FORLIN, L., C. HAUX, L. KARLSSON, and P. RUNN. 1985. Biotransformation enzyme activities in rainbow trout treated with cadmium. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 405-408.

Influence or organic xenobiotics on biotransformation reactions such as hepatic cytochrome P-450 monooxygenase activities in fish, has been more widely studied than influence of inorganic xenobiotics e.g. metal ions. Cadmium has been recognized for its persistence, toxicity and bioaccumulation in aquatic organisms (1). The toxicity of cadmium to fish has been extensively studied and a number of biochemical and physiological effects have been reported (2). However, little information is available on influence of the metal on biotransformation reactions in fish. The purpose of the present study was to investigate the influence of cadmium on biotransformation reactions in liver and kidney of rainbow trout.

Studies were performed on rainbow trout in two separate experiments. In one experiment juvenile, mature male and female trout were used. The fish received two i.p. injections of 0.5 mg/kg cadmium. In the other experiment mature female trout were used. Two groups of fish were exposed to 10 ppb and 100 ppb cadmium in the water.

The i.p. injection of cadmium (sampling after four days) to rainbow trout produced an inhibition in the liver cytochrome P-450 monooxygenase activities tested (ethoxycoumarin-O-deethlase, ethoxyresorufine-O-deethylase and ethylmorphine-N-demethylase activities), whereas no significant effect was observed in the cytochrome P-450 content. In mammalian laboratory animals, depression in cytochrome P-450 monooxygenase activities by administration of cadmium is well known (3). Often associated with this cadmium inhibition is a decrease of the cytochrome P-450 content. In mammals, a role of haem oxygenase has been indicated in the depression of haem proteins by metals (e.g. cadmium) (4).

Many biochemical effects of cadmium result from its ability to bind nucleophilic sites, e.g. sulfhydryl groups. When cadmium was added to the in vitro incubations, it strongly inhibited the monooxygenase activities. This direct effect may indicate that the observed inhibitory responses to treatment with cadmium were due to binding of the metal to nucleophilic sites on the enzymes.

The i.p. administration of cadmium to trout also resulted in marked inhibition in liver and kidney glucuronidation of paranitrophenol (UDPGT) and glutathione conjugation of chlorodinitrobenzene. Also, these enzyme activities were strongly inhibited by addition of cadmium to the in vitro incubations. To produce 50% inhibition, 0.2-0.5 mM cadmium was required which was about two order of magnitude higher concentrations then that required for the monooxygenase activitie (5 µM). However it remains to investigate whether cadmium produce variable in vivo dose-response relationships of the presented enzyme activities in rainbow trout.

It was seen that exposure of female rainbow trout to sublethal concentrations of cadmium for four weeks resulted in a selective response to the metal in that it produced an inhibition of only kidney UDPGT activity but not liver UDPGT activity. It was notable that the livers from fish exposed to 100 ppb contained almost as much of the metal (dry weight basis) as did the kidneys from fish exposed to 10 and 100 ppb. Whether the difference in response to cadmium exposure was due to variable relative abundance of metal (cadmium) binding protein(s) in the two organs, or to un-even distribution of cadmium in different parts of the organ (5) or to other mechanisms of action, has yet to be investigated.

Histological examination of the liver and kidney was performed to ascertain pathological changes as a result of the cadmium exposure via water of the female trout. The hepatocytes were characterized by an increased number of inclusion bodies, a slightly reduced rough endoplasmatic reticulum and a well developed Golgi apparatus. In the nephron the most pronounced alteration was observed within the proximal tubuli. The cellular contours of the tubulus cells could not be clearly distinguished and the luminar caliber of the tubuli was decreased. The cytophasm contained malformed mitochondria. Furthermore eosiniphilic hyaline granulae may be present in a large number which has been suggested to precede necrosis (6).

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FORLIN, L., C. HAUX, L. KARLSSON, and P. RUNN. 1985. Biotransformation enzyme activities in rainbow trout treated with cadmium. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 405-408.

L'influence des xénobiotiques organiques sur les réactions de biotransformation comme les activités de la cytochrome P-450 monoxygénase hépatique chez le poisson, a été plus largement étudiée que l'influence des xénobiotiques inorganiques les ions métalliques, par exemple). Le cadmium a été reconnu pour sa persistance, sa toxicité et sa bioaccumulation dans les organismes aquatiques (1). La toxicité du cadmium pour le poisson a été étudiée sur une grande échelle et un grand nombre d'effets biochimiques et biophysiologiques ont été observés (2). Par contre, on dispose de peu d'information sur l'influence de ce métal sur les réactions de biotransformation chez le poisson. L'objet de notre étude a été de rechercher l'influence du cadmium sur les réactions de biotransformation dans le foie et le rein de la truite arc-en-ciel.

Les études ont été pratiquées sur la truite arc-en-ciel selon deux schémas différents d'expérimentation. Dans l'un, des truites mâles et femelles jeunes et adultes ont été

utilisées. Les poissons recevaient deux injections intrapéritoniales de 0,5 mg/kg de cadmium. Dans l'autre, on s'est servi de truites femelles adultes. Les deux groupes de poissons ont été exposés à 10 ppb et 100 ppb de cadmium dans l'eau.

L'injection intrapéritoniale de cadmium (échantillonnage après quatre jours) pratiquée sur la truite arc-en-ciel a produit une inhibition des activités de la cytochrome P-450 monooxygénase du foie (éthoxycoumarin-O-déséthylase, éthoxyrésorufine-O-déséthylase et éthylmorphine-N-déméthylase), tandis qu'aucun effet significatif n'a été observé sur la teneur en cytochrome P-450. Chez les mammifères de laboratoire, l'inhibition des activités cytochrome P-450 monooxygénase par l'administration de cadmium est bien connue (3). On trouve souvent associée à cette inhibition par le cadmium une baisse du contenu en cytochrome P-450. Chez les mammifères, le rôle de l'hème oxygénase a été noté dans l'inhibition des protéines hémiques par les métaux (cadmium, par exemple) (4).

De nombreux effets biochimiques du cadmium résultent de sa capacité de se fixer sur des sites nucléophiles, par exemple des groupes sulphydryles. Lorsqu'on ajoute du cadmium en cours d'incubation in vitro, il produit une forte inhibition des activités de la monooxygénase. Cet effet direct peut indiquer que les réactions d'inhibition au traitement cadmique seraient dues à la fixation du métal sur des sites nucléophiles des enzymes.

L'administration intrapéritoniale de cadmium à la truite a également entraîné une inhibition marquée de la glycuronidation dans le foie et le rein du paranitrophénol (UDPGT) et de la conjugaison glutathionique du chlorodinitrobenzène. De même, ces activités enzymatiques ont été fortement inhibées par addition de cadmium en cours d'incubation in vitro. Pour produire 50 % d'inhibition, il a fallu 0,2 - 0,5 mM de cadmium représentant environ deux fois l'importance des concentrations demandées pour les activités de la monooxygénase (5 M). Il reste cependant à rechercher si le cadmium produit des relations dose-effet variables in viro de ces activités enzymatiques chez la truite arc-en-ciel.

On a vue que l'exposition de la truite arc-en-ciel femelle à des concentrations sublétales de cadmium pendant quatre semaines a entraîné une réponse sélective au métal, produisant une inhibition de l'activité de l'UDPFT dans le rein, mais non dans la foie. Il est à remarquer que le foie provenant des poissons exposés à 100 ppb contenait presque autant de métal (en poids sec) que les reins provenant des poissons exposés à 10 et 100 ppb. Il reste encore à déterminer si la différence de réponse à l'exposition au cadmium est due à l'abondance relative des protéines à fixation métallique (cadmium) dans les deux organes ou à une distribution inégale du cadmium dans les différentes parties de l'organe (5), ou à d'autres mécanismes d'action.

L'examen histologique du foie et des reins a été pratiqué dans le but de vérifier si des modifications pathologiques étaient dues à l'exposition des truites femelles au cadmium dans l'eau. Les hépatocytes étaient caractérisés par un accroissement du nombre des particules en inclusion, une légère réduction du réticulum endoplasmique et un appareil de Golgi bien développé. Dans le néphron, l'altération la plus prononcée qui fut observée s'est faite dans les tubules proximaux. Les contours cellulaires des cellules tubulaires ne pouvaient être distingués clairement et le calibre de la lumière des tubules était diminué. Le cytoplasme contenait des mitochondries malformées. Enfin, des granules hyalins éosinophiles étaient présents en grande quantité, laissant présager une nécrose (6).

Cette étude a été réalisée grâce au Conseil national de protection de l'environnement de la Suède et partiellement exécutée au Laboratoire de toxicologie des eaux saumâtres, à Studsvik.

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# PHAGOCYTOSIS OF THE INERT SUSPENDED CLAY KAOLIN BY THE GILL EPITHELIUM OF RAINBOW TROUT (SALMO GAIRDNERI)

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GOLDES, S.A. and H.W. FERGUSON. 1985. Phagocytosis of the inert suspended clay Kaolin by the gill epithelium of rainbow trout (Salmo gairdneri). Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 409-410.

Suspended solid levels are increasing in the natural environment as a consequence of soil erosion. The effects of suspended solids on fish reproduction and habitat are well documented, however little work has been done on the direct effect of suspended solids on fish. In this study we have investigated the effect of the inert clay kaolin on the gills of juvenile rainbow trout. The gills were chosen for analysis since this tissue represents an extensive, delicate vascular system which is in direct contact with the external aquatic environment. Gill damage may have far reaching physiological consequences since this organ is the site of gaseous exchange, acid-base regulation, nitrogenous excretion, osmostic regulation and is a frequent site of entry for pathogenic organisms. This study presents the ultrastructural effects of kaolin on gill tissue.

Eight month old rainbow trout were exposed to mean concentrations of 1,017 and 4,87 mg/L kaolin for 32 days. Gills were sampled at 0, 4, 16 and 32 days. The third gill arch from two fish per treatment was processed for transmission electron microscopy at each sample time.

Intracellular membrane-bound, electron dense particles, tentatively identified as kaolin, were found within the branchial filament and lamellar epithelium of all fish exposed to 1,017 and 4,887 mg/L kaolin at 4, 16 and 32 days. The amount of intracellular kaolin was greater at all sample times in fish exposed to 4,887 mg/L kaolin than in those exposed to 1,017 mg/L kaolin. Cells containing kaolin were otherwise ultrastructurally normal.

This study indicates that cells within the gill epithelium may be capable of phagocytosing the inert clay kaolin from the external aquatic environment. Branchial phagocytosis, combined with the extensive gill surface area may therefore represent an important portal of entry for suspended clay particles and possibly other types of particulate material. Although kaolin phagocytes were ultrastructurally normal, other non-inert clays may be capable of causing cellular damage. Clays which are more heavily charged are known to sorb contaminants such as metals and insoluble organic toxicants. Once phagocytosed, sorbed contaminants may react with intracellular constituents. It is therefore possible that phagocytosis of contaminated clays may alter gill structure and function, compromise homeostasis and possibly create opportune conditions for branchial infection by pathogens.

GOLDES, S.A. and H.W. FERGUSON. 1985. Phagocytosis of the inert suspended clay Kaolin by the gill epithelium of rainbow trout (Salmo gairdneri). Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 409-410.

Le niveau des particules solides en suspension augmente dans l'environnement naturel, en fonction de l'érosion des sols. L'effet des particules solides en suspension sur la reproduction des poissons et sur leur habitat a été bien étudié, mais peu de travaux ont été consacrés à l'effet direct des particules solides en suspension sur le poisson. Dans cette étude, nous avons recherché les effets des particules de kaolin inerte sur les branchies de la jeune truite arc-en-ciel. Nous avons choisi d'étudier les branchies, car leur tissu représente un système vasculaire étendu et délicat qui est en contact direct avec l'environnement aquatique extérieur. Les altérations touchant les tissus des branchies peuvent avoir des conséquences physiologiques étendues, car cet organe est le lieu d'échanges gazeux, d'une régulation acide-base, d'excrétion azotée, de la régulation osmotique, et constitue souvent la porte d'entrée des organismes pathogènes. Notre étude sera consacrée aux effets ultra-structuraux du kaolin sur le tissu des branchies.

Des truites arc-en-ciel de huit mois ont été exposées à des concentrations moyennes de 1,017 et 4,887 mg/L de kaolin pendant 32 jours. Des branchies ont été prélevées aux jours 0, 4, 16 et 32. Le troisième arc branchial de deux poissons par traitement a été traité pour examen au microscope électronique par transmission au moment de chaque prélèvement.

Des particules denses aux électrons, liées à la membrane intracellulaire, provisoirement identifiées comme du kaolin, ont été trouvées à l'intérieur du filament branchial et de l'épithélium lamellaire de tous les poissons exposés à 1,017 et 4,887 mg/L de kaolin aux jours 4, 16 et 32. La quantité de kaolin intracellulaire était plus grande au moment de chaque prélèvement chez les poissons exposés à 4,887 mg/L de kaolin que chez ceux exposés à 1,017 mg/L de kaolin. Les cellules contenant du kaolin étaient par ailleurs ultrastructurellement normales.

Notre étude semble indiquer que les cellules qui se trouvent à l'intérieur de l'épithélium des branchies sont capables de phagocyter le kaolin inerte provenant de l'environnement aquatique extérieur. La phagocytose branchiale, combinée à l'étendue de la surface des branchies, pourrait représenter une importante porte d'entrée pour les particules d'argile en suspension et, peut-être, d'autres types de particules. Les phagocytes chargés de kaolin se sont montrés ultrastructurellement normaux, mais il est possible que d'autres argiles non inertes soient capables de provoquer des altérations cellulaires. On sait que certaines argiles plus lourdement chargées sont capables de sorber des contaminants comme les métaux et les toxiques organiques insolubles. Une fois phagocytés, ces contaminants sorbés peuvent réagir avec les constituants intracellulaires. Il est donc possible que la phagocytose des argiles contaminées altère la structure et le fonctionnement des branchies, et compromette ainsi l'homéostase, en créant des conditions favorables à une infection branchiale par des organismes pathogènes.

# TAXONOMY IN AQUATIC TOXICOLOGY - SOME CRUSTACEAN EXAMPLES

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GRIGG, V.M. and P.G. WELLS. 1985. Taxonomy in aquatic toxicology - some crustacean examples. Can. Tech. Rep. Fish. Aquat. Sci. 1368: p. 411.

Animals used in aquatic toxicity experiments are either selected cultured species or wild-caught populations. In both cases, exact identification of species and age-groups of exposed organisms is essential. Modern taxonomy, which included a knowledge of species distributions and often genetic and biochemical factors as well, should be utilized in both the selection and identification of experimental subjects. Cultured species are often aberrant members of their taxa; often they are not native to the areas where toxic conditions may be anticipated. They may also show considerable intraspecific variation. Wild-caught assemblages are natural and local but contain more than one species; if carefully enumerated they can be reliably worked with. This presentation shows new crustacean examples illustrating the importance of taxonomy to environmental toxicology - copepods in a laboratory toxicity study on oilspill dispersants, and ostracods from an organically polluted field site. Both examples reinforce the principle that taxonomy is a key discipline contributing to aquatic toxicology.

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Les animaux utilisés dans des expériences en toxicité aquatique sont ou bien des espèces choisies et cultivées ou bien des populations prises dans la nature. Dans les deux cas, il est essentiel d'identifier exactement l'espèce et les groupes d'âge des organismes exposés. La taxonomie moderne, qui comprend la connaissance de la répartition des espèces et souvent des facteurs génétiques et biologiques, devrait être utilisée à la fois dans la sélection et dans l'identification des sujets d'expérience. Les espèces cultivées sont souvent des membres aberrants de leur taxon; fréquemment, ils ne sont pas nés dans les régions où l'on peut anticiper des conditions toxiques. Ils peuvent également présenter de considérables variations intraspécifiques. Les assemblages pris dans la nature sont naturels et locaux, mais renferment plus d'une espèce; s'ils sont soigneusement dénombrés, ils peuvent constituer un matériel de travail fiable. Notre présentation a pour objet de montrer de nouveaux exemples de crustacés illustrant l'importance de la taxonomie en toxicologie environnementale: des copépodes utilisés dans une étude de toxicité en laboratoire portant sur des produits de dispersion de déversements pétroliers, et des ostracodes provenant d'un lieu naturel pollué par des éléments organiques. Ces deux exemples viennent renforcer le principe selon lequel la taxonomie est une discipline-clé qui contribue à la toxicologie aquatique.

# REFERENCE MIXTURES OF PCB CONGENERS

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GUEVREMONT, R., W.D. JAMIESON, and E. LEWIS. 1985. Reference mixtures of PCB congeners. Can. Tech. Rep. Fish. Aquat. Sci. 1368: p. 413.

As a qualitative and quantitative aid to the practical capillary GC/ECD determination of PCB congeners in marine sediment reference materials CS-1, HS-1 and HS-2, a series of synthetic reference mixtures has been prepared. The mixtures (in iso-octane solvent) contain reliably established levels of selected individual PCB congeners, chosen to represent those prevalent in natural marine sediments, and those believed to be toxic. The sample preparation, tests of homogeneity and tests of the identity of the PCB compounds will be described. Typical GC/ECD and GC/MS data for the reference mixtures will be shown.

GUEVREMONT, R., W.D. JAMIESON, and E. LEWIS. 1985. Reference mixtures of PCB congeners. Can. Tech. Rep. Fish. Aquat. Sci. 1368: p. 413.

On a préparé une série de mélanges de référence synthétiques comme aide qualitative et quantitative à la détermination capillaire pratique par chromatographie en phase gazeuse et détecteur à capture électronique des congénères de BPC dans des matériaux de référence de sédiments marins CS1, HS1 et HS2. Les mélanges (dans un solvant isooctane) contiennent des niveaux convenablement établis de congénères de BPC individuels, sélectionnés en vue de représenter ceux qui prédominent dans les sédiments marins naturels, et ceux que l'on suppose être toxiques. Nous décrivons la préparation de l'échantillon, les tests d'homogénéité et les tests d'identité des composés BPC. Nous indiquons également les données typiques de chromatographie en phase gazeuse et de détection par capture électronique et de chromatographie en phase gazeuse accouplée à la spectrophotométrie de masse pour les mélanges de référence.

# GROWTH AND NUTRIENT UPTAKE INHIBITION IN SELENASTRUM CAPRICORNUTUM SUBJECTED TO DISSOLVED ORGANIC MATTER (DOM) FROM A SECONDARY WASTEWATER EFFLUENT

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LANGIS, R., P. COUTURE, N. MÉTHOT, and J. DE LA NOUE. 1985. Growth and nutrient uptake inhibition in Selenastrum capricomutum subjected to dissolved organic matter (DOM) from a secondary wastewater effluent. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 415-417.

Little is known of the inhibition effects of DOM on primary producers. A better understanding of organic matter-phytoplankton interactions is necessary for the optimization of a tertiary wastewater treatment system using algae: i.e. some investigators have reported growth enhancement, others growth inhibition under the influence of DOM. This study aims at demonstrating the effect of three molecular weight fractions of DOM (> 100K, < 100K and > 10K; < 10K and > 2K) on growth of the green algae Selenastrum capricornutum and on phosphate uptake.

The DOM originates from a secondary wastewater effluent. It is fractionated and washed by a combination of ultrafiltration and diafiltration, using Diaflo membranes. The first and second fractions are submitted to a comparative bioassay experiment, where nutrient supplemented (AAP) and unsupplemented effluent fractions are used as culture media. In addition, the third fraction phosphorus concentration was adjusted to the control level. Algae growth response is measured by cell counts and fluorescence, and nutrient ($PO_4^{-3}$) utilization is followed.

Results show that algae growth rate and $PO_4^{-3}$ uptake are inhibited in the unsupplemented media, while we noted growth rate and $PO_4^{-3}$ uptake enhancement in the AAP supplemented media. Maximum specific growth rates in the unsupplemented media were significantly lower than in the control for all DOM fraction, being incomplete in the intermediate DOM fraction (100K > DOM > 10K).

In the AAP supplemented media, maximum specific growth rate was significantly higher than the control in the largest fraction (DOM > 100K), while being equivalent to the control (although it was reached earlier) in the two other DOM fraction. $PO_4^{-3}$ uptake was significantly faster than in the control in all supplemented media.

It is likely that essential nutrients (other than N and P) complexation by DOM is responsible for growth and $PO_4^{-3}$ uptake inhibition by making some of these unavailable. The stimulation response is more hazardous to explain. Praskah and Rashid (1968) made the hypothesis that cell wall permeability is enhanced under the influence of humic substances.

We also found that there is an inhibition effect linked to organic substances leached from new Diaflo membranes, the manufacturer's washing recommendation being insufficient, and that ultrapure reagent grade water (Milli-Q) is slightly contaminated with organic substances.

We think that since DOM acts on growth and nutrient uptake, it will also affect the nutritive potential of the algae. In further experiments we plan to monitor photosynthetic products (proteins, lipids and sugars) in the cells using a C-14 technique.

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On sait peu de choses des effets d'inhibition provoqués par les MOD sur les producteurs primaires. Il est nécessaire de mieux comprendre les interactions matière organique-phytoplancton lorsqu'on se propose d'optimiser un système de traitement d'eaux usées tertiaires utilisant les algues : certains chercheurs en effet ont observé sous l'influence des MOD une augmentation de croissance, tandis que d'autres notaient une inhibition de croissance. La présente étude a pour but de démontrer l'effet de trois groupes de poids moléculaire de MOD (>100 K, <100 K et >10 K et >2 K) sur la croissance des algues vertes Selenastrum capricornutum et sur la fixation des phosphates.

Les MOD proviennent d'un effluent d'eaux usées secondaires. Ils sont fractionnés et lavés par une combinaison d'ultrafiltration et de diafiltration, au moyen de membranes Diaflo. La première et la deuxième fractions sont soumises à une expérience de dosage biologique comparatif, dans le cadre de laquelle des fractions d'effluent avec et sans substances nutritives (AAP) sont utilisées comme milieu de culture. En plus, les concentrations en phosphore de la troisième fraction sont ajustées au niveau témoin. La réaction de croissance des algues est mesurée par numération cellulaire et fluorescence, et on suit l'utilisation des substances nutritives ($PO_4$-3).

Les résultats montrent que le taux de croissance des algues et la fixation de $PO_4$-3 sont inhibés dans les milieux sans substances nutritives tandis qu'on note dans les milieux avec AAP une augmentation du taux de croissance et de la fixation de $PO_4$-3. Les taux de croissance spécifique maximaux dans les médias non additionnés ont été significativement inférieurs à ceux des témoins pour toutes les fractions des MOD, tout en étant incomplets dans la fraction intermédiaire des MOD (100 K > MOD > 10 K).

Dans les milieux avec AAP, le taux de croissance spécifique maximal a été significativement plus élevé que le témoin dans la plus forte fraction (MOD > 100 K), tout en étant équivalent au témoin (mais en l'atteignant plus tôt) dans les deux autres fractions des MOD. La fixation de $PO_4$-3 a été significativement plus rapide que dans le témoin dans tous les milieux avec AAP.

Il est probable que la complexation des substances nutritives essentielles (autres que N et P) par les MOD est responsable de l'inhibition de la croissance et la fixation de $PO_4$-3, en rendant certaines de ces substances non disponibles. L'effet de stimulation est plus difficile à expliquer. Prakash et Rashid (1968) ont avancé l'hypothèse selon laquelle la perméabilité de la membrane cellulaire serait renforcée sous l'influence des substances humiques.

Nous avons également noté qu'il se produit un effet d'inhibition lié aux sustances organiques extraites des nouvelles membranes Diaflo, les recommendations de lavage du fabricant s'étant révélées insuffisantes, et que l'eau ultrapure de qualité réactif (Milli-Q) est légèrement contaminée par des substances organiques.

Nous pensons que, du fait que les MOD agissent sur la croissance et la fixation des nutriants, elles affecteront également le potentiel nutritif des algues. Dans des expériences ultérieures, nous projetons de rechercher les produits de photosynthèse (protéines, lipides et sucres) dans les cellules, au moyen d'une technique au carbone 14.

# EVALUATION OF A RADIOMETRIC ASSAY FOR METALLOTHIONEIN SYNTHESIS IN CADMIUM EXPOSED MUSSELS (MYTILUS EDULIS)

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LOBEL, P.B. and J.F. PAYNE. 1985. Evaluation of a radiometric assay for metallothionein synthesis in cadmium exposed mussels (Mytilus edulis). Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 419-420.

Metallothionein induction can be a useful indicator for the presence of some biologically active heavy metals. We have evaluated the feasibility of using a radiometric technique to detect the induction of metallothionein and metallothionein-like proteins in cadmium exposed mussels. Preliminary trials included studies with gill tissue but hepatopancreatic tissue was found to be more sensitive. Suitable assay conditions for hepatopancreatic tissue included homogenization in 1.15% KCl and centrafugation at 16 000 g for 1 h. The resulting supernatant was treated with mercuric chloride labelled with mercury-203 and then treated with trichloroacetic acid (TCA) to precipitate the high molecular weight cytosolic proteins. The levels of activity remaining in the TCA supernatant were subsequently measured. In mussels exposed to cadmium, the radioactivity of the TCA supernatant was elevated indicating that metal-binding protein had been induced. Induction was readily detectable in mussels exposed to 8 ppb cadmium, a level actually lower than levels reported for some polluted environments. This indicates that the method can be a useful adjunct to more complex methods of metallothionein analysis and is sensitive enough for use in field studies. However, the assay should not be used in the presence of high levels of copper since copper ions were found to markedly interfere with the analysis. Other metals tested, including aluminum, cadmium, lead, manganese and zinc showed little or no interference. Two advantages of this method over gel chromatography are as follows: (a) large scale screening programmes can be accomodated, (b) only small amounts of tissue are required (<1 g).

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L'induction de métallothionéine peut être considérée comme un indicateur utile de la présence de certains métaux lourds biologiquement actifs. Nous avons évalué la possibilité d'utiliser une technique radiométrique destinée à détecter l'induction de la métallothionéine et des protéines analogues dans des moules exposés au cadmium. Parmi les essais préliminaires, nous avons étudié les tissus branchiaux, mais les tissus hépatopancréatiques se sont révélés plus sensibles. Les conditions d'essai appropriées pour les tissus hépatopancréatiques comprennent une homogénéisation dans du KCl à 1,15 % et la centrifugation à 16 000 g pendant une heure. La fraction surnageante a été traitée avec du chlorure mercurique marqué au mercure 203, puis traitée à l'acide trichloroacétique (ATC), de façon à faire précipiter les protéines cytosoliques à poids moléculaire élevé.

Les niveaux d'activité restant dans l'ATC surnageant ont été ensuite mesurés. Dans les moules exposées au cadmium, la radioactivité de l'ATC surnageant s'est montrée élevée, indiquant qu'une protéine à fixation métallique avait été induite. L'induction a été facilement décelable chez les moules exposées à 8 parties par milliard de cadmium, niveau effectivement plus faible que les niveaux observés dans certains milieux pollués. Ces observations indiquent que la méthode employée peut être utilement associée à des méthodes plus complexes d'analyse de métallothionéine et qu'elle est suffisamment sensible pour des études sur le terrain. Mais elle ne doit pas être employée en présence de niveaux élevés de cuivre, car on a observé que les ions cuivre perturbent considérablement l'analyse. D'autres métaux mis à l'essai, l'aluminium, le cadmium, le plomb, le manganèse et le zinc, ont eu une influence faible ou nulle. Les avantages que présente cette méthode par rapport à la chromatographie sur gel sont les suivants : a) des programmes de dépistage à grande échelle peuvent être mis sur pied; b) la méthode ne demande que de faibles quantités de tissus (<1 g).

# DIETARY UPTAKE OF MERCURY IN WALLEYE AND PIKE

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MATHERS, A. and P. JOHANSEN. 1985. Dietary uptake of mercury in walleye and pike. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 421-422.

It is known that methylmercury is accumulated by fish via two pathways: (1) directly from the water through the gills and (2) from their diet. The proportion of the body burden of mercury which is accumulated from the diet is unknown for field situations. This paper describes the quantification of the amounts of mercury consumed with the diets of pike (Esox lucius) and walleye (Stizostedion vitreum) in Lake Simcoe, Ontario.

In this study the diet of both walleye and pike has been documented. The diet of the walleye is characterized by a strong preference for smelt (69% by weight). The pike has a more varied diet and they consume much less smelt (16% by weight). Food consumption rates and the concentration of mercury in the prey items have been determined. Smelt are the most highly contaminated prey item. These data have been used to estimate mercury consumption rates. The estimated rates have then been compared to the observed levels of mercury contamination in the pike and walleye populations. Relative to pike, walleye have a more highly mercury contaminated diet and the concentrations of mercury observed in their bodies was higher at all ages. The proportion of the body burden of mercury which is accumulated from the diet is discussed.

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On sait que le méthylmercure s'accumule dans le poisson selon deux mécanismes : 1) directement à partir de l'eau à travers les branchies et 2) à partir de l'alimentation. La proportion de la charge corporelle de mercure qui s'accumule à partir du régime alimentaire est inconnue dans des situations sur le terrain. Dans cet article, nous décrivons la quantification du mercure consommé dans le régime alimentaire du brochet (Esox lucius) et du doré (Stizostedion vitreum) dans le lac Simcoe, en Ontario.

Au cours de notre recherche, nous avons étudié le régime alimentaire du doré et du brochet. L'alimentation du doré se caractérise par une forte préférence pour l'éperlan (69 % en poids), tandis que le brochet a une alimentation plus variée et consomme relativement moins d'éperlan (16 % en poids). Les taux de consommation alimentaire et la concentration de mercure dans les proies consommées ont été déterminés. Parmi celles-ci, l'éperlan est le plus contaminé. Ces données ont été utilisées pour estimer les taux de consommation de mercure. Puis ces taux ont été comparés aux niveaux observés de contamination mercurielle dans les populations de brochets et de dorés. Par comparaison avec le brochet, le doré a une alimentation nettement plus contaminée en mercure, et les

concentrations de ce métal dans son organisme sont plus élevées, quel que soit l'âge. Enfin, nous analysons la proportion de la charge corporelle de mercure qui s'accumule à partir de l'alimentation.

# MICROINJECTION OF RAINBOW TROUT EMBRYOS: AN *IN VIVO* CARCINOGENESIS ASSAY

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METCALFE, C.D. and R.A. SONSTEGARD. 1985. Microinjection of rainbow trout embryos: an *in vivo* carcinogenesis assay. Can. Tech. Rep. Fish. Aquat. Sci. 1368: p. 423.

An *in vivo* carcinogenesis assay was developed in which nanogram quantities of carcinogens were injected into rainbow trout embryos. Liver tumors were induced in trout one year after exposure to carcinogens. Neoplasms were inducted by single injections of 13 and 25 ng per egg of aflatoxin $B_1$, 500 ng per egg of 7,12 dimethylbenzanthracene, and 250 ng per egg of 2-anthramine. Over 70% of ($^3$H)benzo(a) pyrene injected into eggs was retained in hatched embryos, 120 hours post-injection. Exogenous activation of test compounds using rat-liver microsome preparation (S-9) may have increased the incidence of liver tumors in fish injected with aflatoxin and DMBA. This assay has been used to evaluate the carcinogenicity of industrial effluent extracts.

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Une étude de la carcinogénèse *in vivo* a été mise au point, comportant l'injection dans des embryons de truite arc-en-ciel de quantités infimes d'agents cancérigènes (nanogrammes). Un an après exposition aux produits cancérigènes, des tumeurs ont été observées chez les truites au niveau du foie. Les néoplasmes ont été induits par simple injection de 13 et 25 ng par oeuf, d'aflatoxine $B_1$; 500 ng par oeuf, de 7,12 diméthylbenzanthracène; et 250 ng par oeuf, de 2-anthramine. Plus de 70 % de $^3$Hbenzo(a)pyrène injecté dans les oeufs a été retenu dans les embryons développés, 120 heures après injection. Une activation exogène des produits d'essai au moyen de préparation de microsome de foie de rat (S-9) peut avoir augmenté l'incidence des tumeurs hépatiques chez les poissons injectés avec l'aflatoxine et la DMBA. Cet essai a été utilisé pour évaluer la carcinogénécité des extraits d'effluents industriels.

# BIOAVAILABILITY AND TOXICITY OF SIX SYNTHETIC PYRETHROIDS TO CHIRONOMUS TENTANS LARVAE IN SEDIMENT-WATER SYSTEMS

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MUIR, D.C.G., G.P. RAWN, B.E. TOWNSEND, and W.L. LOCKHART. 1985. Bioavailability and toxicity of six synthetic pyrethroids to Chironomus tentans larvae in sediment-water systems. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 425-426.

Pyrethroid insecticides are hydrophobic chemicals that sorb readily to suspended solids and sediments if introduced into aquatic systems. Field studies using small ponds have indicated that these compounds can persist in bottom sediments for more than 16 weeks. In view of the high acute toxicity of synthetic pyrethroids to many aquatic species information is needed on their availability to sediment-dwelling animals. This study was designed to measure bioconcentration of pyrethroids and survival of Chironomus tentans larvae in sediments containing concentrations of the insecticides similar to those found in field experiments.

Chironomus tentans larvae were exposed to six $^{14}$C-labelled pyrethroids in sediment and in water above sediment (sediment:water ratio, 1:10). Sediments were spiked at 5 and 50 ug/kg (wet weight) and concentrations of each compound were monitored in water throughout a 24 or 48 hr exposure period. Elimination of radioactivity by larvae was followed over a 96 hr period in clean sand-water systems following the exposure.

Concentrations of each pyrethroid in solution in water above sediment were generally <0.1 ug/L above river and pond sediments (silty-clays) and <1.0 ug/L above sand in the 50 ug/kg sediment exposures. Survival of larvae exposed to cis-permethrin, cis-cypermethrin and deltamethrin was low in water above sand containing 5 or 50 ug/kg. After 24 hrs exposure in water above sand, two distinct groups of compounds could be discerned, those with concentration factors (CFs) ranging from 100 to 125 (trans-permethrin, trans-cypermethrin and fenvalerate) and those with CFs ranging from 200 to 300 (cis-cypermethrin, cis-permethrin and deltamethrin). Larvae exposed in sediment had generally higher CFs than those exposed in the same container in water above sediments (in a screened cup). CFs for larvae exposed in river and pond sediments were generally lower than those exposed in sand. Elimination of radioactivity by larvae was rapid with half-lives of 15-20 hrs and was well described by a single compartment model.

We concluded that pyrethroid insecticides were readily accumulated from a variety of sediments by Chironomus larvae. The accumulation appeared to be water mediated and directly related to the sediment-water equilibrium established by each chemical in the exposure vessel. Bioavailability was further demonstrated by the mortality of larvae exposed to the cis-isomers in sediments treated at 5 and 50 ug/kg.

MUIR, D.C.G., G.P. RAWN, B.E. TOWNSEND, and W.L. LOCKHART. 1985. Bioavailability and toxicity of six synthetic pyrethroids to Chironomus tentans larvae in sediment-water systems. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 425-426.

Les insecticides pyréthroides sont des produits hydrophobes qui se sorbent rapidement aux particules solides en suspensions et aux sédiments si on les introduit dans des systèmes aquatiques. Les études sur le terrain menées dans de petites mares ont indiqué que ces produits peuvent persister dans les sédiments inférieurs pendant plus de 16 semaines. Si l'on considère l'extrême toxicité aigue des pyréthroides de synthèse pour un grand nombre d'espèces aquatiques, il est nécessaire de recueillir le maximum de renseignements sur leur disponibilité pour les animaux qui vivent dans les régions sédimentaires. Notre étude a pour objet de mesurer la bioconcentration des pyréthroides et la survie des larvaes de Chironomus tentans dans des sédiments contenant des concentrations d'insecticides analogues à celles qu'on observe dans des expériences sur le terrain.

Des larves de Chironomus tentans ont été exposées à six pyréthroides marqués au carbone 14 dans des sédiments et dans l'eau surnageant les sédiments (rapport sédiment: eau = 1:10). Les sédiments ont été traités par 5 et 50 µg/kg (poids humide) et les concentrations de chaque produit ont été mesurées dans l'eau sur une période d'exposition de 24 ou 48 heures. L'élimination de la radioactivité par les larves a été suivie sur une période de 96 heures dans des systèmes eau-sable propre, après l'exposition.

Les concentrations de chaque pyréthroide en solution dans l'eau au-dessus des sédiments ont été d'une façon générale inférieures à 0,1 µg/L au-dessus des sédiments de rivière et de mare (vase argileuse) et inférieures à 1,0 µg/L au-dessus du sable dans les expositions aux sédiments à 50 µg/kg. La survie des larves exposées à la cis-perméthrine, la cis-cyperméthrine et la deltaméthrine a été faible dans l'eau au-dessus du sable contenant 5 ou 50 µg/kg. Après 24 heures d'exposition dans l'eau au-dessus du sable, on a pu discerner deux groupes distincts de corps, ceux avec des facteurs de concentration (FC) variant entre 100 et 125 (trans-perméthrine, trans-cyperméthrine et fenvalerate) et ceux avec des facteurs de concentration compris entre 200 et 300 (cis-cyperméthrine, cis-perméthrine et deltaméthrine). Les larves exposées dans les sédiments ont eu généralement des facteurs de concentration plus élevés que celles qui étaient exposées dans le même contenant dans l'eau au-dessus du sédiment (dans un tube examiné). Le facteur de concentration des larves exposées dans les sédiments de rivière et de mare a été d'une façon générale inférieur à celui des larves exposées dans le sable. L'élimination de la radioactivité par les larves a été rapide, avec des demi-vies de 15-20 heures et bien décrite par un modèle à compartiment unique.

En conclusion, on peut dire que les insecticides pyréthroides ont été facilement accumulés à partir d'une variété de sédiments par les larves de Chironomus. L'accumulation semble avoir été entrainée par l'eau, en relation directe avec l'équilibre sédiment-eau établi pour chaque produit chimique dans le contenant d'exposition. La biodisponibilité a été démontrée par ailleurs par la mortalité des larves exposées aux isomères cis, dans les sédiments traités à raison de 5 et 50 µg/kg.

# CADMIUM CYCLING BETWEEN WATER, SEDIMENT AND BIOTA IN AN ARTIFICALLY CONTAMINATED MUD FLAT ON THE NORTH SEA

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PROSI, F., D.H. LORING, and G. MULLER. 1985. Cadmium cycling between water, sediment and biota in an artificially contaminated mud flat on the North Sea. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 427-428.

Very little is known about the physical, chemical, and biological interactions that take place during heavy metal contamination of tidal mud flats.

To study such interactions, two Bremerhaven caissons were used to investigate the interactions of cadmium (Cd) with sea water, particulate matter (SPM), sediments, and biota in $13^2$m enclosed systems on a tidal mud flat of the outer Jade Bay (North Sea).

Cadmium as a chloride was continuously injected into one of the caissons so as to maintain a concentration of 100 ug/l Cd in sea water in the inflowing water of each tidal cycle for 22 days. The other caisson, about 50 m away, was used as an environmental control without the addition of Cd.

During the experiment, each component of the system (SPM, sediment, biota) was sampled at selected intervals inside each caisson and in the outside environment. Additional parameters measured included water and sediment temperature, salinity, tidal heights, and grain size of the sediment samples.

In the laboratory, Cd was determined by ASV (water) and AAS (SPM, sediments, and biota). Transmission electron microscopy (TEM) was also used to observe Cd deposits in animal tissues ($H_2S$) method).

The results show that the SPM, sediments, and biota become progressively contaminated with Cd over the course of the experiment. They illustrate that studies of enclosed systems such as this provide invaluable information on the way in which interacting processes work to govern the behavior of contaminants on tidal mud flats and how the biota in such an environment reacts to this contamination.

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On sait très peu de choses sur les interactions physiques, chimiques et biologiques qui se produisent pendant la contamination par les métaux lourds des vasières tidales.

Afin d'étudier ces interactions, deux caissons de Bremerhaven ont été utilisés pour étudier les interactions du cadmium avec l'eau de mer, en particulier les produits particulaires (SPM), les sédiments et le biote dans des systèmes clos de 13 $m^2$ situés sur des vasières tidales dans la partie extérieure de Jade Bay (mer du Nord).

Du cadmium sous forme de chlorure a été injecté de façon continue dans un des caissons afin de maintenir une concentration de 100 µg/L de Cd dans l'eau de mer pénétrant à chaque cycle de marée, pendant 22 jours. L'autre caisson, à une distance d'environ 50 m, a été utilisé comme témoin environnemental sans addition de Cd. Durant les expériences, chaque composant des systèmes (SPM, sédiment, biote) a été soumis à des prélèvements à intervalles choisis à l'intérieur de chaque caisson et dans l'environnement extérieur. Parmi les autres paramètres mesurés, citons la température de l'eau et celle des sédiments, la salinité, la hauteur des marée et la granulométrie des échantillons de sédiments.

Au laboratoire, le cadmium a été mesuré par redissolution anodique (eau) et par spectroscopie d'absorption atomique (SPM, sédiment et biote). On a également utilisé le microscope électronique à transmission, pour observer les dépôts de Cd dans les tissus animaux (méthode $H_2S$).

Les résultats obtenus montrent que SPM, sédiment et biote deviennent progressivement contaminés par le cadmium au cours du déroulement de l'expérience, ce qui prouve que les études de systèmes clos fournissent des renseignements inestimables sur la manière dont se produisent les interactions qui gouvernent le comportement des contaminants dans les vasières tidales et sur la façon dont le biote de cet environnement réagit à la contamination.

# THE RELATIONSHIP BETWEEN THREE POTENTIAL PATHOGENS AND POLLUTION INDICATOR MICROORGANISMS IN NOVA SCOTIAN COASTAL WATERS

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ROBERTSON, W.J. and R.S. TOBIN. 1985. The relationship between three potential pathogens and pollution indicator microorganisms in Nova Scotia coastal waters. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 429-430.

Fifteen stations, in two estuaries, along the Northumberland Strait of Nova Scotia were examined between June and September 1981 for a relationship between the concentrations of commonly monitored fecal indicator bacteria and the potential pathogens *Candida albicans*, *Pseudomonas aeruginosa* and *Vibrio parahaemolyticus*. Increased densities of these three microorganisms were usually associated with high densities of indicator bacteria while *C. albicans* and *P. aeruginosa* occur in human fecal wastes, *V. parahaemolyticus*, indigenous to the marine environment, positively responds to elevated nutrient levels in sewage. There is also some evidence that these bacteria survive as long or longer in marine waters than the common indicator bacteria. While membrane filtration techniques for the enumeration of *C. albicans* and *P. aeruginosa* proved satisfactory, a *V. parahaemolyticus* membrane filtration method lacked specificity and was supplemented by a most-probable-number method. In marine recreational and shellfish waters these three organisms could complement fecal coliforms and fecal streptococci as indicators of human fecal contamination.

ROBERTSON, W.J. and R.S. TOBIN. 1985. The relationship between three potential pathogens and pollution indicator microorganisms in Nova Scotia coastal waters. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 429-430.

Quinze stations, situées dans deux estuaires le long du détroit de Northumberland en Nouvelle-Écosse, ont été étudiées entre les mois de juin et septembre 1981, à la recherche d'une relation entre les concentrations de bactéries indicatrices de contamination fécale généralement employées, et les agents pathogènes potentiels suivants: *Candida albicans*, *Pseudomonas aeruginosa* et *Vibrio parahaemolyticus*. Des accroissements de densité de ces trois microorganismes ont été généralement associés avec de fortes densités de bactéries servant d'indicateurs, tandis que *C. albicans* et *P. aeruginosa* se trouvant dans les matières fécales d'origine humaine, *V. parahaemolyticus* vivant dans l'environnement marin réagissent positivement à une élévation des niveaux de substances nutritives dans les effluents d'égouts. On a également observé que ces bactéries survivent aussi longtemps ou plus longtemps dans l'eau de mer que les bactéries employées communément comme indicateurs. Les techniques de filtration sur membrane employées pour la numération de *C. albicans* et de *P. aeruginosa* se sont révélées satisfaisantes mais la méthode de filtration sur membrane appliquée à *V. parahaemolyticus* a manqué de spécificité et a dû être associée à la méthode du nombre le plus probable. Dans les eaux

récréatives et d'élevage des coquillages, ces trois organismes ont servi de complément aux coliformes fécaux et aux streptocoques fécaux, comme indicateurs de contamination par les matières fécales de l'homme.

# DETERMINATION OF THE DISTRIBUTION OF TOXICITY IN FISH TISSUE USING THE NEMATODE BIOASSAY

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SAMOILOFF, M., R. PULAK, and T. BOGAERT. 1985. Determination of the distribution of toxicity in fish tissue using the nematode bioassay. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 431-435.

A bioassay method using the free-living nematode Panagrellus redivivus has been shown to be a rapid method for determining the overall toxic effects of compounds (Samoiloff et al, 1980) and complex mixtures (Samoiloff et al, 1983). The bioassay is used to assess the type of toxic effect produced by the tested material, with the following priority of effects:

1. Lethality, producing 100% death in the tested population.

2. Semilethality, producing a significant (but not 100%) proportion of death in the tested population.

3. Developmental inhibition, producing a decrease in the overall rate of development, indicating inhibitory effects on the overall metabolism.

4. Genotoxicity, producing a specific inhibition of those events that require extensive gene activity and macromolecular biosynthesis. This effect is distinct from mutagenesis, although many mutagens exert a genotoxic effect on the P. redivivus bioassay.

Since the bioassay is performed under controlled laboratory conditions, it can be equally applied to freshwater, marine or gaseous samples. The bioassay is primarily used to rank the toxic effects of a series of samples, ranking the toxic effects of the samples. One application, previously reported, is the determination of the fractions of sediments which contain the greatest toxic effects, to focus on the real toxicity of the sediments.

Here we report on the utility of the P. redivivus bioassay in locating the types and spatial distribution of toxic materials that bioaccumulate in fish tissues. Samples of brain, fat, and muscle tissue of size-paired red-suckers (Moxostoma).

SAMOILOFF, M., R. PULAK, and T. BOGAERT. 1985. Determination of the distribution of toxicity in fish tissue using the nematode bioassay. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 431-435.

Une méthode de dosage biologique utilisant le nématode vivant en liberté nommé *Panagrellus redivivus* s'est montrée un moyen rapide de déterminer les effets toxiques globaux des produits isolés (Samoiloff et coll., 1980) et des mélanges complexes (Samoiloff et coll., 1983). Cette méthode de dosage biologique est utilisée pour évaluer le type d'effet toxique produit par le matériel testé, dans l'ordre de priorité d'effet suivant:

1. létalité, entraînant 100 % de décès dans la population d'essai;
2. semi-létalité, produisant une proportion importante (mais différente de 100 %) de mortalité dans la population d'essai;
3. inhibition du développement, entraînant une diminution du taux global de développement, traduisant des effets inhibiteurs sur le métabolisme général;
4. génotoxicité, entraînant une inhibition spécifique des processus qui demandent une importante activité des gènes et de la biosynthèse macromoléculaire. Cet effet est différent de celui de la mutagénèse, bien que certains mutagènes exercent un effet génotoxique sur le dosage biologique par le *P. redivivus*.

Le dosage biologique étant effectué dans des conditions contrôlées de laboratoire, il peut s'appliquer de la même façon à l'eau douce, à l'eau de mer ou à des échantillons gazeux. L'objectif essentiel du dosage biologique est de classer par rangs les effets toxiques d'une série d'échantillons en les évaluant de façon individuelle. Une application, qui a fait l'objet d'une communication précédente, consiste à déterminer quelles fractions de sédiments contiennent la plus grande activité toxique, de façon à concentrer les activités sur la toxicité réelle des sédiments.

Nous avons rassemblé ici nos observations sur l'utilité du dosage biologique au *P. redivivus* pour déterminer les types et la distribution spatiale des matériaux toxiques qui font l'objet d'une bioaccumulation dans les tissus des poissons. Ont été étudiés des prélèvements du cerveaux, des graisses et des tissus musculaires de suceurs rouges (*moxostomas xxx*) rassemblés par taille.

# INTRODUCTION

A bioassay method using the free-living nematode *Panagrellus redivivus* has been shown to be a rapid method for determining the overall toxic effects of compounds (Samoiloff *et al*, 1980) and complex mixtures (Samoiloff *et al*, 1983). The bioassay is used to assess the type of toxic effect produced by the tested material, with the following priority of effects:

1. *Lethality*, producing 100% death in the tested population.
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4. *Genotoxicity*, producing a specific inhibition of those events that require extensive gene activity and macromolecular biosynthesis. This effect is distinct from mutagenesis, although many mutagens exert a genotoxic effect on the *P. redivivus* bioassay.

Since the bioassay is performed under controlled laboratory conditions, it can be equally applied to freshwater, marine or gaseous samples. The bioassay is primarily used to rank the toxic effects of a series of samples, ranking the toxic effects of the samples. One application, previously reported, is the determination of the fractions of sediments which contain the greatest toxic effects, to focus on the real toxicity of the sediments.

Here we report on the utility of the *P. redivivus* bioassay in locating the types and spatial distribution of toxic materials that bioaccumulate in fish tissues. Samples of brain, fat, and muscle tissue of size-paired red-suckers (*Moxostoma macrolepidotum*) and pike (*Esox lucius*) were obtained from sites upstream and downstream from a dam which blocks migration. For each species, three upstream fish were matched to three downstream fish, and the patterns of *P. redivivus* growth were examined for downstream fish tissues relative to the tissue of the paired upstream fish. The results are shown in Tables 1 and 2.

Four parameters are determined in the test. Survival (%S) is not significantly reduced in any of the tissue samples. Two general growth parameters (P1 and P2) also show no significant effects in any of the fish tissues. The parameter (P3) requiring extensive gene activity and macromolecular synthesis is specifically inhibited by the fat tissue of all downstream suckers, and in the fat tissue of the largest pike. This inhibition is significant to the 0.05 level.

These results demonstrate the presence of a toxic material present downstream (but not upstream) of the dam, which is specifically accumulated in fatty tissue. The presence of the toxic material in all tested downstream suckers, but in only the largest pike, indicates that the material was relatively recently introduced into the ecosystem, and that the material is probably associated with sediments.

TABLE 1 THE P. REDIVIVUS BIOASSAY ON TISSUES FROM DOWNSTREAM PIKE (ESOX LUCIUS) RELATIVE TO UPSTREAM PIKE

| FISH | TISSUE | %S | P1 | P2 | P3 | E | FITNESS |
|---|---|---|---|---|---|---|---|
| 1 | muscle | N | N | N | N | - | 96 |
| 2 | muscle | N | N | N | N | - | 97 |
| 3 | muscle | N | N | N | N | - | 98 |
| 1 | fat | N | N | N | *** | G | 73 |
| 2 | fat | N | N | N | N | - | 106 |
| 3 | fat | N | N | N | N | - | 100 |
| 1 | brain | N | N | N | N | - | 99 |
| 2 | brain | N | N | N | N | - | 103 |
| 3 | brain | N | N | N | N | - | 106 |
| pooled | muscle | N | N | N | N | - | 97 |
| pooled | fat | N | N | N | N | - | 99 |
| pooled | brain | N | N | N | N | - | 103 |

N = No significant effect
*** = inhibition significant to the $P<0.005$ level

- = No overall effect
G = Genotoxic effect

Fitness is a measure of the overall growth and survival of test animals relative to their upstream controls. A fitness of 100 indicates growth and survival similar to the upstream controls, while decreased values indicate decreased fitness.

Tables 1 and 2 also present the types of toxic effect observed (G represents significant geotoxicity), and a quantitative measure (referred to as fitness) of the growth and survival of downstream fish relative to upstream fish.

This study shows the utility of the P. redivivus bioassay for determining the types of toxicity associated with complex environmental samples.

TABLE 2 THE P. REDIVIVUS BIOASSAY ON TISSUES FROM DOWNSTREAM SUCKERS (MOXOSTOMA MACROLEPIDOTUM) RELATIVE TO UPSTREAM PIKE

| FISH | TISSUE | %S | P1 | P2 | P3 | E | FITNESS |
|---|---|---|---|---|---|---|---|
| 1 | muscle | N | N | N | N | - | 97 |
| 2 | muscle | N | N | N | N | - | 98 |
| 3 | muscle | N | N | N | N | - | 100 |
| 1 | fat | N | N | N | *** | G | 97 |
| 2 | fat | N | N | N | *** | G | 77 |
| 3 | fat | N | N | N | *** | G | 83 |
| 1 | brain | N | N | N | N | - | 100 |
| 2 | brain | N | N | N | N | - | 99 |
| 3 | brain | N | N | N | N | - | 101 |
| pooled | muscle | N | N | N | N | - | 100 |
| pooled | fat | N | N | N | *** | G | 83 |
| pooled | brain | N | N | N | N | - | 100 |

N = No significant effect
*** = inhibition significant to the $P<0.005$ level

- = No overall effect
G = Genotoxic effect

Fitness is a measure of the overall growth and survival of test animals relative to their upstream controls. A fitness of 100 indicates growth and survival similar to the upstream controls, while decreased values indicate decreased fitness.

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# THE EFFECT OF OIL-CONTAMINATED PREY ON THE ENERGETICS OF PINK SALMON FRY

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SCHWARTZ, J.P. 1985. The effect of oil-contaminated prey on the energetics of pink salmon fry. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 437-438.

Previous experiments with pink salmon fry feeding on oil-contaminated prey demonstrated that fry feeding rate decreases as the concentration of oil-in-prey increases. Fry growth rate decreased when the fry were reared on oil-contaminated prey, and reduced fry growth was attributed to a reduction in feeding rate. However, petroleum hydrocarbons absorbed from water or ingested by fish are quickly detoxified through energy-requiring metabolic pathways. The energy needed to detoxify petroleum hydrocarbons depletes energy available for growth. The objective of ongoing research is to determine the energetic cost of feeding on oil-contaminated prey by subtracting the energy required for maintenance (energy loss from respiration and excretion) from the energy ingested (net caloric intake after absorption efficiency is measured) to find the net energy available for growth.

The energy budget of pink fry was monitored daily while they were fed live brine shrimp nauplii contaminated with aromatic hydrocarbons by exposure to the water soluble fraction (WSF) of Cook Inlet crude oil. Fry feeding rate, oxygen consumption, ammonia excretion, and fecal production were simultaneously measured to calculate fry energy budget. Two oil-in-prey concentrations were tested (0.5 ug/g and 5 ug/g) and a second control group was fed near-starvation rations to measure fry energetic with reduced food intake independently of exposure to oil-in-prey.

Fry oxygen consumption decreases when food intake is reduced, and absorption efficiency of food increases. Fry fed oiled food experience reduced feeding rate but absorption efficiency decreases and ammonia excretion increases, resulting in a net loss of energy available for growth beyond what is lost from reduced feeding rate. The reduced growth rate of fry fed oil-contaminated prey results primarily from a reduction in energy intake. Additional energy for growth is lost in order to detoxify and excrete petroleum hydrocarbons.

SCHWARTZ, J.P. 1985. The effect of oil-contaminated prey on the energetics of pink salmon fry. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 437-438.

Des expériences antérieures pratiquées sur des alevins de saumon rose nourris au moyen de proies contaminées par le pétrole ont démontré que son taux d'alimentation décroissait à mesure qu'augmentait la concentration de pétrole dans la proie. La croissance des alevins décroît lorsqu'ils sont nourris de proies contaminées par le pétrole, et cette diminution de croissance a été attribuée à une réduction du taux d'alimentation.

Cependant, les hydrocarbures pétroliers absorbés à partir de l'eau ou ingérés par le poisson sont rapidement détoxifiés au moyen de mécanismes métaboliques demandant de l'énergie. L'énergie utilisée pour détoxifier les hydrocarbures de pétrole provoque une perte d'énergie aux dépens de la croissance. L'objectif de la recherche en cours est de déterminer le coût énergétique de l'alimentation à partir de proies contaminées par le pétrole en soustrayant l'énergie nécessaire pour l'entretien de la vie (dépense d'énergie de la respiration et de l'excrétion) de l'énergie captée par alimentation (calories reçues après mesure du rendement d'absorption), afin de trouver l'énergie nette disponible pour la croissance.

Le bilan énergétique des alvenins de saumon rose a été surveillé chaque jour tandis qu'il était nourri de nauplius d'artemia contaminé par des hydrocarbures aromatiques par exposition à la fraction soluble dans l'eau du pétrole brut de l'inlet Cook. Le taux d'alimentation des alevins, leur consommation d'oxygène, l'excrétion d'ammoniac et la production fécale ont été mesurés simultanément en vue de calculer leur bilan énergétique. Les concentrations de pétrole dans la proie ont été mesurées (0,5 µg/g et 5 µg/g) et un deuxième groupe témoin a reçu des rations de presque-famine, de façon à mesurer la capacité énergétique des jeunes sujets soumis à une réduction de l'alimentation indépendamment de toute exposition au pétrole.

La consommation d'oxygène des jeunes sujets a décrû à mesure que diminuait l'alimentation, et à mesure qu'augmentait le rendement d'absorption de l'alimentation. Au cours de l'expérience d'alimentation contaminée de pétrole, on a noté une réduction du taux d'alimentation, une diminution de l'absorption, avec une augmentation de l'excrétion d'ammoniac, le tout produisant une perte d'énergie nette pour la croissance, au-delà du niveau de perte provenant de la réduction du taux d'alimentation. Le taux de réduction de croissance du jeune poisson recevant une alimentation contaminée par le pétrole résulte essentiellement d'une réduction d'énergie fournie. Une proportion supplémentaire d'énergie est perdue aux dépens de la croissance, dans le but de détoxifier et d'excréter les hydrocarbures pétroliers.

# SUB-LETHAL TESTS WITH FISH: THEIR PERTINENCE FOR ECOTOXICOLOGICAL EVALUATIONS IN LOCAL AND REGIONAL IMPACT STUDIES

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THELLEN, C., R. VAN COILLIE, and M. BIENVENUE. 1985. Sub-lethal tests with fish: their pertinence for ecotoxicological evaluations in local and regional impact studies. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 439-440.

Impact studies of pollution, whether the result of effluent discharge or a micropollutant, bring forth two considerations. First, the usefulness of experimental work in the laboratory is well known. Second, the choice of susceptible parameters, likely to be expressed in the natural milieu, is to be recommended. The tally of different researches has permitted us to appraise favourably certain tests with fish:

- Avoidance, the reaction of a fish confronted with a non favourable sensory stimulus in its milieu.
- Swimming behavior, the consequences of physiological pertubations caused by a toxic environment.
- Examination at gill segments, the preferential site of action of pollutants and the precursor to different toxic reactions.

With the aim of maximizing the interpretation of results, it is advisable to be selective in the experimental approach, notably in the choice of species of fish, dilution water and sample. This synthesis review deals with the problems of methodology, the results and the ecotoxicological interpretations of the prementionned tests.

THELLEN, C., R. VAN COILLIE, and M. BIENVENUE. 1985. Sub-lethal tests with fish: their pertinence for ecotoxicological evaluations in local and regional impact studies. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 439-440.

Des études des effets de la pollution, résultant d'un rejet d'effluent ou d'un micropolluant, conduisent à deux types de considérations. Premièrement, l'utilité du travail d'expérimentation au laboratoire est bien connue. Deuxièmement, on recommande le choix de paramètres de sensibilité pouvant s'exprimer dans le milieu naturel. Une étude comparative des différentes recherches nous a permis d'évaluer favorablement certains tests portant sur des poissons:

- évitement, la réaction d'un poisson confronté avec un stimulus sensoriel non favorable dans son milieu;

- le comportement natatoire, conséquence de perturbations physiologiques provoquées par un environnement toxique;
- examen des saignements branchiaux, lieu préférentiel d'action des polluants, et précurseur des différentes réactions toxiques.

Afin de maximiser l'interprétation des résultats, il est conseillé d'être très sélectif dans la méthode expérimentale, notamment dans le choix des espèces de poisson, l'eau de dilution et l'échantillonnage. Cette revue de synthèse traite des problèmes de méthodologie, des résultats et de l'interprétation écotoxicologique des tests mentionnés plus haut.

# A MARINE FISH SPECIES EXPOSED TO CRUDE OIL: EFFECTS ON FEEDING, SELECTED PHYSIOLOGICAL PARAMETERS AND LIVER LIPIDS FOLLOWING A FOUR-WEEK RECOVERY PERIOD

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WILLIAMS, U.P., J.W. KICENIUK, and A.C. DEY. 1985. A marine fish species exposed to crude oil: effects on feeding, selected physiological parameters and liver lipids following a four-week recovery period. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 441-442.

Cunners (Tautogolabrus adspersus) were exposed to a 50-200 ppb water extract of Hibernia Crude oil. The exposure period lasted 8 1/2 weeks, followed by a four week recovery period. Upon termination of the experiment, organ somatic indices and condition factors (weight/length$^3$) were calculated. Liver somatic index was the only physical parameter significantly affected by oil exposure. Hematocrits and hemoglobins were determined periodically during the study. Total liver lipids and phospholipids were determined gravimetrically following extraction and separation by chromatography. Significantly higher phospholipids were found in both sexes of exposed fish. Lipid class and fatty acids were determined by TLC and GLC respectively. In exposed fish, wax exters were significantly decreased and free fatty acids were significantly increased. Triglycerides and diglycerides were unaffected and steryl esters were not detectable in experimental fish. Fatty acid series $C_{18:4}$ and $C_{21:5}$ were significantly lower and $C_{16}$, and $C_{16:4}$ and $C_{18:1}$ were significantly higher in both experimental sexes. $C_{20:1}$ and $C_{22:6}$ were significantly lower in experimental females and $C_{16:1}$ was significantly lower in experimental males. These findings indicate that oil exposure, even after a four-week recovery period, produces altered pattern of lipid metabolism which can persist for some time.

WILLIAMS, U.P., J.W. KICENIUK, and A.C. DEY. 1985. A marine fish species exposed to crude oil: effects on feeding, selected physiological parameters and liver lipids following a four-week recovery period. Can. Tech. Rep. Fish. Aquat. Sci. 1368: pp. 441-442.

Des tanches tautogues (Tautogolabrus adspersus) ont été exposées à un extrait aqueux de pétrole brut d'Hibernia à 50-200 parties par milliard. La période d'exposition a duré huit semaines et demie, suivies d'une période de rétablissement de quatre semaines. À la fin de l'expérience, les indices somatiques de différents organes et les facteurs de condition (poids/longueur$^3$) ont été calculés. L'indice somatique du foie a été le seul paramètre physique significativement affecté par l'exposition au pétrole. Durant l'étude, les hématocrites et les taux d'hémoglobine ont été mesurés périodiquement. Les lipides totaux du foie et les phospholipides ont été déterminés gravimétriquement après extraction et séparation par chromatographie. On a noté des phospholipides significativement

plus élevés dans les deux sexes des poissons exposés. Lipides et acides gras ont été déterminés par chromatographie en couches minces et chromatographie gaz-liquide. Chez le poisson exposé, les esters de paraffine étaient significativement diminués tandis que les acides gras libres marquaient une augmentation significative. Triglycérides et diglycérides n'étaient pas modifiées et les esters stéryliques n'étaient pas décelables dans le poisson examiné. Les séries d'acides gras $C_{18:4}$ et $C_{21:5}$ étaient significativement inférieures, tandis que les séries $C_{16}$, $C_{16:4}$ et $C_{18:1}$ étaient significativement plus élevées dans les deux sexes. $C_{20:1}$ et $C_{22:6}$ étaient significativement inférieures chez les femelles et $C_{16:1}$ était significativement inférieur également chez les mâles. Ces résultats indiquent que l'exposition au pétrole, même après une période de rétablissement de quatre semaines, produit une modification du métabolisme des lipides qui peut persister pendant un certain temps.

# SUMMARIES OF OTHER RECENT MEETINGS ON ECOTOXICOLOGY

# REPORT ON THE "INTERNATIONAL SYMPOSIUM ON ECOTOXICOLOGICAL TESTING FOR THE MARINE ENVIRONMENT (MARTOX)"

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From September 12 through September 14, 1983 an international Symposium on marine ecotoxicology was convened at the State University of Ghent in Belgium by the Laboratory for Biological Research in Aquatic Pollution.

The principal goal of this Symposium which was sponsored by the Commission of the European Communities and attended by 200 experts of 20 countries was to evaluate the existing scientific knowledge on marine ecotoxicological testing and to identify the needs for further research and development.

The scientific program of the Convention comprised reviews presented by invited experts, round tables on specific themes, and poster sessions with experience papers.

Twenty-one reviews have been presented by renowned experts from America and Europe, as well on the state of the art of marine ecotoxicology in different countries, as on marine ecotoxicological tests with different types of organisms.

Lectures were also presented on special topics such as "Experimental procedures for hazard assessment in the marine environment" and "Future trends in marine ecotoxicology".

In the poster exhibit 41 experience papers on display covering various topics of marine ecotoxicology complemented in more detail the themes treated by the reviewers.

The last day of the convention was entirely devoted to three round tables on the following subjects:

1) Standardization of marine ecotoxicological test methods
2) Financial implications of marine hazard assessment
3) Predictive value of marine ecotoxicological tests.

A synthesis of the reports presented by the rapporteurs of the round tables during the closing session is outlined below.

## Standardization of marine ecotoxicological test methods

1. Standardization of marine bioassays is highly desirable not to impose specific test recipes, but to provide (to whoever needs or want them) well described procedures which guarantee highly reproducible results.

2. Standardization of test methods is moreover necessary at the national and international level for the implementation of laws and conventions dealing with the protection of the marine environment.

3. Test protocols of marine bioassays available to date are usually not described in enough detail, especially from the chemical point of view.

   Basic principles of "Good Laboratory Practice" are often not respected by those who carry out the tests; this lead to variabilities in the results which have often been attributed to variation in the sensitivity of the test-species used.

4. Selection of test-species for standardized tests should be made according to the final goal of the bioassay:

   - for screening of chemicals a limited number of "all round" key species should be selected

   - for monitoring of discharges, locally important species shall be used to increase the relevancy of the results for the specific case situation.

5. Intercalibration exercizes (Round Robin tests) are very important to check the reliability and the reproductibility of test protocols proposed as standard bioassays.

   The number of such exercizes performed so far is extremely small as is the number of participating labs; the outcome of the marine Round Robin tests carried out to date has been in most cases discouraging.

   One test method, however, seems to have successfully passed thorough examination at the international level. It concerns the short term Artemia nauplii LC50, better known as the ARC-test (Artemia Reference Center test), which has been the subject of an intercalibration exercize with participation of 70 European and 12 North American labs*.

6. Training of scientists and technicians in laboratories specialized in particular test methodologies can improve the reproductibility of results to a considerable extent and consequently be very beneficial for standardization.

7. More sophisticated tests, such as chronic or bioaccumulation bioassays, - although less susceptible to standardization - should therefore not be disregarded in the general framework of hazard assessment of chemicals in the marine environment.

**Financial implications of marine hazard assessment**

1. The number of tests performed and the degree of complexity determine the cost but also the quantity of information obtained.

2. The "high" costs of testing are relative because for new chemicals they are low in comparison to production and marketing costs and extremely low when compared to the expenses one faces if "accidents" occur with a toxic chemical, or after the release of a hazardous waste product.

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* Due to a long postal strike in Canada from where the materials had to be send out, finally only 12 out of the 125 interested institutes in North America could turn in their results in time.

3. The costs of ecotoxicological testing can be reduced in several ways:

   1) by a better use of the existing data, especially with regard to the relationship between structure and properties of a chemical and its effects (QSAR = quantitative-structure -activity relationship),

   2) by a justified choice of test methods and tiers in relation to the degree of complexity of the problem and the degree of hazard,

   3) by the development of better criteria to determine the necessity to pursue or stop testing at a certain point.

4. Future efforts (in time as well as in money and efforts) should focus more on the validation of existing test protocols than on the development of new ones.

5. "Ecologically relevant" tests, such as (field) microcosm tests are not always as expensive as one usually thinks.

6. A multidisciplinary approach for marine testing, especially with regard to the physical and chemical aspects will result in better cost-effective procedures.

**Predictive value of marine ecotoxicological test**

The purpose of the predictive value of marine bioassays is to allow anticipating and solving environmental problems primarily through laboratory tests. The ultimate optimal predictive tool would be a short-term single-species test to predict community responses in the field. It is, however, probable that this goal will never be completely achieved.

1. There is an urgent need for a useful data basis. Often the analysis of old data and their reinterpretation is an unrewarding exercize because important factors may have been overlooked in the experiments leading to the data. Predictive capabilities should be based first on the development of a testable hypothesis followed by an appropriate experimental design and data acquisition.

   For many years, scientists seem to have been more preoccupied with the development of their own bioassay methods to generate additional data on the toxicity of chemicals, without an implicit or explicit strategy for predictability.

2. As far as criteria verification is concerned, current water-quality criteria are a type of verification which is limited to the type of species tested, and cannot readily be extrapolated to communities. There is a need to develop "site-specific" or "zone-specific" criteria that take the species' biological variability and the bio-availability of the chemicals in the receiving environment into account.

3. The artificial conditions of testing in the laboratory should be taken into consideration. On the one hand are species in nature usually not living in the optimal conditions under which bioassays are carried out in the laboratory and on the other hand are test species often stressed by the artificial conditions under which they are kept.

4. There is a use and a need for safety factors to extrapolate laboratory data to real situations in the natural environment. The figures for safety factors should be determined empirically and not be selected arbitrarily.

   Application factor seems to be a more appropriate term than safety factor since the latter implies a level of protection which is not precisely defined.

5. With regard to hazard assessment as a predictive strategy, it has been underlined that ecotoxicologists should be aware of the limits of their biological test methods and endeavor to improve the predictive value of their bioassays. In this regard, an integration of chemistry and biology is of paramount importance.

   In the first place a thorough knowledge and understanding of chemical exposure is essential; secondly the fate of the contaminant must be considered to define the habitat, the species and the types of tests needed to determine the biological impact of the chemical.

All the papers presented during the Symposium (reviews as well as experience papers) and the syntheses of the three round tables with the conclusions and recommendations will be published in a special volume:

"ECOTOXICOLOGICAL TESTING FOR THE MARINE ENVIRONMENT
PROCEEDINGS OF AN INTERNATIONAL SYMPOSIUM"

This volume, which should be available by mid-summer 1984 will be published jointly by the Laboratory for Biological Research in Aquatic Pollution of the State University of Ghent (Belgium) and the Institute for Marine Scientific Research (Belgium).

Distribution will be handled by the European Mariculture Society, Prinses Elisabethlaan 69, 8401 Bredene, Belgium, to which all orders have to be addressed:

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# REPORT ON THE WORKSHOP ON QSAR IN ENVIRONMENTAL TOXICOLOGY

16-18 August 1983, McMaster University, Hamilton, Ontario

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## INTRODUCTION

In the last five to ten years, our awareness of and concern over environmental contaminants has risen substantially. With that, the number of known contaminants in the ecosystem has multiplied as well. No longer is our concern restricted to a few classes such as PCB's and chlorobenzenes, we now know of the occurrence of many other chlorinated products, such as polychlorinated styrenes, dibenzo-p-dioxins, di-benzofurans, anthracenes, phenanthenes, pyrenes, anilines, napthalines. In addition, there are more and more non-halogenated compounds being found, for example organotin derivatives which are widely used in agricultural and industrial applications.

The great increase in known contaminants has shifted the focus from identification to assessment of impact and hazard. However, biological tests are equally costly and time consuming and cannot be done on all compounds of interest. We therefore have to search for alternate means, such as mathematical models to estimate or predict the effects of certain compounds. This requirement brings us to the art/science of QSAR, quantitative structure-activity correlations. QSAR has been pioneered by C. Hansch and is now widely used in the development of new drugs, pesticides and other products.

This workshop on QSAR in Environmental Toxicology was intended to bring together scientists from various disciplines working in the general fields of environmental contaminants, their pathways and effects with special emphasis on structure-activity relationships.

## WORKSHOP FORMAT

### Physical Conditions

The workshop was spread over three full days, with the afternoon of the second day for outings and individual contacts and discussion. McMaster University provided an excellent forum with accommodation, food services, and recreational facilities at reasonable cost and close proximity to the meeting room. The meeting room itself, had a seating capacity of approximately 30 attendees. Of particular interest is the u-shaped layout of the meeting room (FIGURE 1) which provides for the speaker to be the centre of

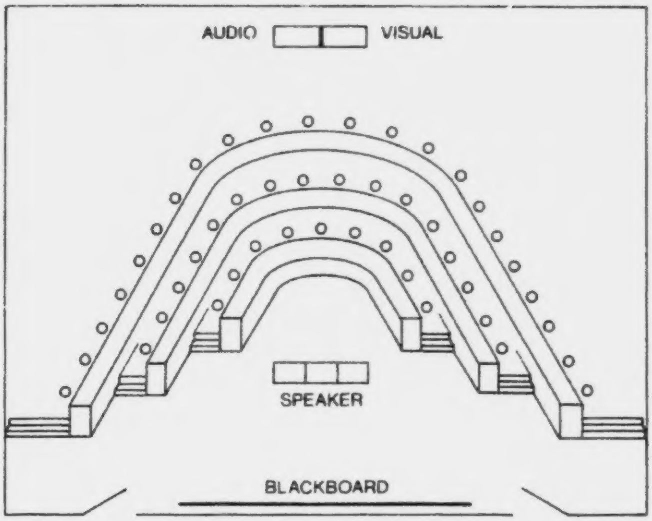

FIGURE 1 LAYOUT OF CONFERENCE ROOM

the audience, thus providing an intimate atmosphere, good audibility and view from all seats.

As no concurrent sessions were held, each participant had opportunity to attend all lectures. This format apears most useful as it eliminates the breaking up of audiences into discipline-oriented factions. On the contrary, it provides for interdisciplinary discussion and a good deal of scientific stimulation through the representation of different points of view.

### Schedule

Lectures were between 15 and 45 min. duration in no particular order. As no concurrent sessions were held, the various lengths did not affect the organizational aspects. One, most beneficial feature appeared to be a period of discussion following each presentation. The length of this discussion period was dependent on the length of the preceeding presentation and was usually one third of the latter. Discussion periods were therefore geared to the overall content and information transmitted by the presentation. Indeed, this format was found to be very beneficial by most participants. Almost without exception, each discussion period resulted not only in the clarification of points presented but evolved into a forum for the frank exchange of ideas, opinions, and experiences from all participants.

## PROCEEDINGS

Most of the papers presented at the workshop have been submitted in full for the proceedings, which will become available in April 1984 at an approximate cost of $35.00

from D. Reidel Publishing Company, Dordrecht, Holland. The title of the book will be "QSAR IN ENVIRONMENTAL TOXICOLOGY". Anyone wishing to obtain further information may contact me or the publisher.

## CONTENTS

Alice Bobra et al., University of Toronto, Toronto:

The Acute Toxicities of oils, hydrocarbons and chlorinated hydrocarbons to Daphnia is well correlated with the aqueous solubility ($C_L$) of these compounds. A representative equation for Daphnia is:

$$\log 48\text{-}LC50 = -0.53 + 0.60 \log C_L$$
$$n = 18;\ r^2 = 0.77$$

In Chu et al., Health and Welfare Canada, Ottawa:

A very detailed study on the effects of the three tetrachlorobenzene isomers 1,2,3,4-("1"); 1,2,3,5-("2"); and 1,2,4,5-$Cl_4$ ("3"); several differences in relative activity of the isomers were noted. The much higher sublethal toxicity of "3" is due to its much stronger bioaccumulation and retention and slower metabolism relative to the other isomers:

LD50: $2 < 3 < 1$
Bioaccumulation ($^{14}C$): $1 \sim 2 << 3$
Chronic toxicity: $1 \sim 2 << 3$

George Dixon, et al., University of Waterloo, Waterloo:

Report on a study to evaluate the potential use of plasma proteolytic enzyme leucine amino naphthylamidase to assess acute/sublethal toxicant impact on rainbow trout. On the basis of data for p-cresol and carbon tetrachloride, the method appears to be a promising indicator.

William Dunn et al., University of Illinois, Chicago:

A demonstration of the applicability of the SIMCA pattern recognition method to both the identification of sources and the characterization of contaminant mixtures on the example of PCB's in sediments and biota.

Dieter Freitag et al., GSF, Munich, FRG:

A comprehensive review of the "Environmental Hazard Profile", tests and results n approximately 100 compounds. Shows the relative potentials for bioaccumulation in several aquatic species, for retention in rats, biodegradation by activated sludge, and photodegradation. Introduction to the sphere fragmentation process for the characterization of compounds for structure-activity models.

Donald Hart, IEC Beak Consultants Ltd., Mississauga:

Demonstration of a new mutagenicity test on the examples of ethyl methanesulfonate (EMS), ethylnitroso urea (ENU), and diethyl nitrosamine. The test uses male South African clawed frogs (Xenopus laevis) and shows recessive lethal effects in haploid embryos after paternal treatment with effective mutagens, such as EMS and ENU.

Peter V. Hodson et al., GLFRB, Burlington:

LD50's, measured by intraperitoneal injection of contaminants to fish, are correlated with oral LD50's and aqueous exposure LC50's, the traditional measure of contaminant toxicity. The speed and efficiency of the injection type bioassay makes it a valuable tool for QSAR studies of known or potential environmental contaminants.

Reiner Koch, Institute of Hygiene, Gera, GDR:

A study on the possibilities and limits of QSAR in ecotoxicology with special emphasis on topological indices, such as the connectivity index. Good correlations were obtained for a variety of fish and algae bioconcentration factors with two or more dimensional models. A representative equation for chlorinated aliphatics, benzenes, and phenols toxicity to guppy is:

$$\log LC50 = 4.73 - 0.75 \log P - 0.59 \log N + 0.17\ ({}^{2}X^{v})$$

Klaus Kaiser et al., NWRI, Burlington:

Review of a collaborative study on the QSAR of various chlorophenol, chlorobenze, and para-substituted phenol toxicities by intraperitoneal injection (IPLD50) and other bioassays. Representative equations are:

Chlorobenzenes:

rainbow trout $p\ (IPLD50) = 1.71 + 0.20 \log P$
$n = 11;\ r^2 = 0.78$

Chlorophenols:

rainbow trout $p\ (IPLD50)\ \ -2.18 + 0.59 \log P$
$n = 5;\ r^1 = 0.89$

rainbow trout $p\ (IPLD50) = -6.98 + 1.17 \log P + 0.40\ pKa$

Para-substituted phenols: $n = 5;\ r^2 = 0.98$

rainbow trout $p\ (IPLD50) = 0.21 + 0.12\ MR + 1.54\ R$
$n = 8;\ r^2 = 0.94$

trout cell attachment bioassay (Bols et al., 1983):
$p\ (EC50) = -2.23 + 0.53 \log P$
$n = 8;\ r^2 = 0.94$

Kazimiera Kwasniewska et al., NWRI, Burlington:

QSAR's of chloroaniline toxicities to four strains of yeast. A representative equation is:

Rhodotorula rubra:

$$\log(1/EC50)\ \ 1.63 + 1.19 \log P - 0.11 (\log P)^2\ n = 7;\ r^2\ \ 0.91$$

Sheldon Lande et al., 3M Environmental Lab., St. Paul:

Total molecular surface areas (TSA) were used to estimate aqueous solubilities of hydrocarbons. The method promises to be of interest with compounds being part of new classes of contaminants.

Tom Lander et al., Health Designs, Inc., Rochester:

An overview of existing QSAR models on human toxicological potentials and presentation of a new model for aquatic contaminants, based on a very comprehensive data collection. The results indicate good predictive power for biodegradation of compounds with or without significant degradation rates.

Gerald LeBlanc, EG&G Bionomics, Wareham:

Acute toxic effects of chloro and methylphenols, 1,2,4-trichlorobenzene, and a phthalate ester to fathead minnows were determined. In conjunction with published toxicity data and structure coefficients, acute-chronic toxicity relations are developed for chlorobenzenes and phenols. In contrast to the phenols, the chronic toxicity of benzenes increases more rapidly with increasing chlorination than their acute toxicity.

Donald Mackay et al., University of Toronto, Toronto:

Review of recent advances in the use and intercorrelations of bioconcentration factors, partitioning and equilibrium coefficients. There is a continuing need for the determination of many physical-chemical and toxicological properties of known and suspected contaminants.

Larry Newsome et al., US-EPA, Washington:

A comparison of measured and predicted fish toxicities of five classes of non-reactive, non-electrolyte herbicides based on their octanol/water partition coefficients. Thirtyseven chemicals and 49 fish tests are investigated. Several herbicides were found to be considerably less toxic than predicted, an effect which is interpreted in terms of limited water solubility.

Barry Oliver, NWRI, Burlington:

Bioconcentration factors of halogenated compounds are well correlated with their octanol/water partition coefficients (log P). However, limitations of this concept are observed for compounds with log P >5, where the time to reach equilibrium may be well over 100 days. Acute and chronic toxicity determinations of such materials likely suffer from the same effects.

Juan Ribo et al, NWRI, Burlington:

QSAR's for chloroanilines are presented. Relationships of Microtox toxicity with the octanol/water partition coefficients and with toxicity to *Rhodotorula rubra* yeast are shown:

$$p\,(30EC50) = -0.04 + 0.57 \log P$$

$$p\,(I50) = -0.13 + 0.61\, p\,(30EC50)$$

Wayne Schultz et al., University of Tennessee, Knoxville:

QSAR's of over 20 aromatic amines, pyridines, and nitroaromatics are developed for the growth impairment of the ciliate *Tetrahymena pyriformis*. The best model found is:

$$\log BR = -\ 1.63 \quad 0.83 \log Kow$$

Vladimir Zitko, Biological Station, St. Andrews:

Overview of recent developments of QSAR in environmental sciences, limits and future directions. New structural descriptors, such as various topological indexes have opened new ways to provide information on compounds with no or few measured parameters. Vapor pressure correlations are useful for predictions of the distribution of chemicals in the environment. Work should now be focussed on the relationships between concentrations in organs and tissues, with toxicity, and with transfer rates. New mathematical techniques, such as pattern recognition analyses may become more important.

## CONCLUSIONS

There is a continuing need for the measurement of physical, chemical data, particularly on compounds representative of new classes and those where extreme values are expected.

Acute toxicity data and, for certain compounds with high partition coefficients, even chronic toxicity data may be incorrect due to kinetic control (bioconcentration equilibrium not reached).

Topological indices appear to be good parameters for QSAR's; they can always be calculated from molecular structures.

Quantitative structure-activity correlations appear to work well for compounds with octanol/water partition coefficients in the $10^2$ to $10^5$ region, more work is needed to verify models outside of this range.

There is too much emphasis on halogenated compounds. New studies should emphasize compounds with various functional groups, heterocyclic and reactive compounds.

Quantitative structure-activity relationships have a critically important role in environmental toxicology, however they must not be used as substitute for experimental work and must not be abused.

# SUMMARY SESSION

P.G. Wells, Chairman

# THE CASE FOR A MOVE TO PATHOGNOMONIC RESEARCH

Mike Gilbertson (Dept. of Fisheries and Oceans, Ottawa)

During the past thirty-five years there has been an increasing involvement of the Canadian Government in the investigation and regulatory control of pollution of the aquatic environment to protect fish, fish habitat and fisheries. This involvement has its jurisdictional basis in the British North America Act and in the Fisheries Act. In preparing regulations, enforcement and other control actions there has been an implicit premise that damage to fish or fisheries can be demonstrated by measurement of environmental levels of pollutants and interpolation of the results of contolled toxicity studies or bio-assays undertaken in the laboratory using pure compounds or samples of effluent. In addition, any person who lays a change under the Fisheries Act must show that the "deposit" of the "deleterious substance" was into a "water frequented by fish".

This premise has recently come under challenge not only in the courts but also in negotiations with industry on existing and proposed regulations under the Fisheries Act. This premise seems to have been necessary because it is frequently extremely difficult to observe effects on fish, particularly sublethal effects, in the field. It is even more difficult to demonstrate a causal relationship between the presence of a pollutant and an observed effect. Thus the interpolation and a reliance on expert witnesses has been necessary as a best approximation in the absence of direct evidence of harm.

Does the premise still hold or have the conditions now changed? There are several examples of instances where this premise has been challenged and thus the kinds of investigation that government organizations need to undertake to demonstrate causality of damage may need to be re-examined.

In Regina v Great Canadian Oil Lands Ltd. in the District Court of Alberta, Edmonton, 1978, the company was acquitted of a charge of permitting the deposit of a deleterious substance in the Athabasca River. The court found that there was no evidence of any harmful effects on the river, nor was there any evidence of deleterious substances in the seepage samples collected from the site despite evidence of acute lethality of the seepage samples to fish under laboratory conditions. Further, the Crown's tests had utilized species of fish not present in the Athabasca River or only rarely so, and these data were deemed insufficient to prove that the seepages were deleterious. This case is of particular interest because it not only shows the necessity for careful work on the environmental pathway whereby the pollutant enters aquatic habitat but also the necessity for information on both effects of the pollutant under laboratory conditions on relevant species and demonstration of actual damage to fish or fisheries.

Recent negotiations between the Federal Government and industry concerning amendments to regulations under the Fisheries Act concerning metal mining and pulp and paper production have been marked by demands by industry that government should present evidence that industrial operations are causing deleterious effects on fish or fisheries. When initial regulations were being developed under the Fisheries Act during the early 1970s industry was not under the same economic pressure now imposed by the austerity of the recession. To a large degree government has been unable to produce sound and persuasive cases on which further regulations can be promulgated with the concurrence of economically beleaguered industries.

The final example concerns acid rain and the preparation of an international agreement on the reduction of sulphur emission. In the development of the "Memorandum of Intent on Transboundary Air Pollution" between the United States and Canada, there was extensive information on the sources of sulphur dioxide emissions, the concentrations in precipitation at various localities, trend data concerning the effects of acidity on fish and fish reproduction under controlled laboratory conditions. The case was however relatively weak concerning the incidence of actual damage in the environment and even weaker concerning the prediction of the extent and severity of the likely damage during the next twenty to thirty years. A quotation from the report concerning evaluation of the evidence of damage to fish populations in the Adirondack Region of New York captures the prevailing skepticism of the present U.S. administration. "It is difficult to evaluate exactly how many fish populations have been lost from Adirondack waters as a result of acidification. 180 Adirondack ponds that formerly sustained brook trout populations no longer support such populations. It has not however been formally demonstrated that all (or most of) these population extinctions occurred as a result of acidic deposition". No matter what scientists, administrators or activist groups may think about the politics of using scientific skepticism to avoid imposition of expensive controls, the quotation in effect has changed the rules of evidence by challenging the original premise. By challenging the premise it necessarily also changes the kinds of work that fish-habitat researchers undertake because laboratory experimentation and chemical analysis of field samples will be insufficient evidence.

The argument against having to "formally demonstrate" causality is that in most cases the extent of damage will be unacceptable by the time that the necessary work has been undertaken. There are several examples where causality was inferred or predicted but was insufficient to effect appropriate controls until a formal demonstration was made and a wild population had crashed or was rendered unfit for human consumption. These examples include the widespread extirpation of several predatory birds brought about by the use of organochlorine insecticides, reproductive failure of fish-feeding birds on Lake Ontario likely caused by chlorinated dibenzo p-dioxins and furans and closure of local inland and marine fisheries because of metals (particularly mercury), polynuclear aromatic hydrocarbons and organochlorine compounds.

What data are needed to "formally demonstrate" the relationship between an observed effect and a suspected causal agent? National and international agencies frequently meet to produce long lists of the diverse kinds of data needed to assess chemicals and other pollutants. Fortunately, the lists can be simplified into a 2x3 matrix. One side of the matrix (Figure 1) is comprised of two simple ideas derived from Paracelsus and classical toxicology; exposure (dose) and effects (toxicity). The second side of the matrix concerns the three distinct kinds of information that provide the evidence for exposure and effects. These are i) controlled experimental studies usually undertaken in the laboratory on laboratory animals using individual pollutants, ii) field surveys, sampling and observations undertaken on wild populations exposed to various environmental pollutants under various uncontrolled environmental conditions, and iii) commercial surveys of the quantities of the pollutant manufactured, used and released through various routes to the environment.

Most scientists can relatively rapidly find where their work fits into the matrix. They tend to produce information either on effects or on exposure and tend to work on controlled laboratory experiments or on field surveys or are concerned with effluent analysis of chemical composition or toxicity. The process has now been challenged by the

| Exposure | | Effects |
|---|---|---|
| Controlled Laboratory Experimentation | Chemodynamics Pharmacodynamics | Toxicity |
| Field surveys, sampling and observations | Environmental levels and trends | Epidemiology (Epizootiology) |
| Sources of Release | Quantity and concentration released | Effluent Toxicity (bioassay) |

FIGURE 1 MATRIX OF KINDS OF STUDIES NEEDED FOR FORMAL DEMONSTRATION OF TOXICOLOGICAL CAUSALITY

new requirement to produce all six kinds of evidence including field-effects data otherwise known as epidemiology or, for animals, epizootiology.

The number of demonstrated cases of pollutants causing damage to fisheries is relatively small particularly for subtle and sublethal effects. Most cases have been concerned with acute accidental releases such as oil spills. There have been a number of cases in which fisheries have been closed because of contamination. But there seem to be few cases in which fisheries biologists were able to show a causal connection between the use of a chemical and damage to the resource. The use of DDT in the 1950s and early 1960s for spruce budworm control in the New Brunswick forests caused the subsequent demise of the salmonid stocks in the Mirimichi River. Elemental phosphorus caused widespread fish mortality in Placentia Bay, Newfoundland from the plant at Long Harbour.

There are a few other cases where incidents of disease have been observed and where the presence of chemicals is suspected but where the causal relationship has not been "formally demonstrated". Examples include the abnormal incidence of gonadal tumors in carp and papillomas in suckers in the Great Lakes and the exposure of those fish to organochlorine compounds and polynuclear aromatic hydrocarbons. Cod taken outside Halifax, Nova Scotia had an abnormal incidence of liver pathology that might have been related to the content of PCB. The number of observed incidents of diseases in fish that may have a chemicals etiology is still fairly small. At the present time we are uncertain as to whether this reflects the rarity of their occurrence or the proportion of funding that has been allocated to this line of investigation.

Epizootiology is not a well developed branch of fisheries scientific inquiry, particularly for chemical pathogenic agents. It is however well developed in verterinary medicine (including aquaculture) and wildlife management.

Medical science was enormously aided in the last century by the formulation of criteria that could be used to show that certain microorganisms were causally related to specific diseases. These criteria were codified into a series of postulates by Koch and have been repeatedly used to "formally demonstrate" the role of pathogenic organisms in human and animal diseases.

I have not seen any comparable set of postulates for chemically induced diseases but an analogous set can be formulated.

I think that most scientists are not, unnaturally, daunted by the complexity of the multifactorial agents that might be involved in epizootiological work on natural populations. Most retreat back to the relative safety of the laboratory where they can produce unequivocal results using genetically-known strains of laboratory organisms exposed to pure compounds under controlled laboratory conditions. This is relatively safe from a career standpoint since it predictably produces publications of a high quality. It does not however, advance our knowledge of what is actually occurring in the environment. As long as government and university scientists, activist groups and politicians are up against industry, both here in Canada and in other nations, this is not going to be sufficient.

To make a complete and well-balanced scientific case on which rational regulatory action can be used there is a priority requirement for the development of a symbiotic relationship between laboratory experimentalists and field observers. Too frequently in the past there has been an antipathy between the approaches; one group was accused of lacking scientific rigor, the other of lacking pragmatism and applicability of their results. Generally however, the experimentalists have won out with the consequence that there is an enormous literature on what pollutants do but almost nothing on what they have done.

It is time to put on the southwester and waders and to go to polluted fish habitat to observe and document what is actually happening to fish in the real world. It is no longer enough to document exposure, we have now been forced also to formally demonstrate effects in the environment.

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